A NOVEL TRANSDERMAL PATCH CONTAINING LAMIVUDINE AND *INVITRO - INVIVO* CHARACTERIZATION

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Submitted by

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Under the guidance of

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This work is original and has not been submitted earlier for the award of any other degree or diploma of this or any other university.

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CONTENTS

1.0 ABSTRACT

The purpose of the work was to formulate and evaluate the matrix transdermal patches of Lamivudine for the controlled delivery of the drug in the body. The patches were prepared by the solvent casting method using Span-80 as a permeation enhancer. The results of the preliminary trials indicate the drug: polymers ratio affected the characteristics of the patches. This patches were prepared in different drug: polymer (Lamivudine:HPMC and Lamivudine:EC) ratios of 1:2.5, 1:5, without permeation enhancers and with permeation enhancers, The drug polymer interaction was investigated by FTIR and the results indicated no incompatibility. Lamivudine patches were evaluated for various parameters like thickness, folding endurance, percentage moisture loss, percentage moisture absorption, drug content uniformity, stability studies, *in vitro* skin permeation, skin irritation test, statistical analysis by ANOVA, *in vivo* studies and *invitro/invivo* . The prepared transdermal patches were subjected to *in vitro* drug permeation study by using rat skin in Phosphate Buffer pH 7.4 for 24 hrs. The comparative statistical analytical data (ANOVA) showed 'p' value < 0.0001which suggest that the prepared formulations are extremely significant for transdermal delivery. The formulation (FPE_2) ratio 1:5 of (Lamivudine:HPMC) patch with permeation enhancer showed best result among all formulation. This formulation (FPE_2) was subjected to *in-vivo* studies by using rat. For *in vivo* studies patches were administered transdermally to rats, while a standard solution of 10µg/ml was used as a control, and collected the plasma at different time interval and analyzed by HPLC technique, and carried out the *in-vitro in-vivo correlation*. Thus showed a near correlation for *in-vitro and in-vivo* studies.

2.0 INTRODUCTION

Transdermal drug delivery is the non-invasive delivery of medications from the surface of skin-the largest and most accessible organ of human body- through its layers, to the circulatory system. TDDS offers many advantages over conventional injection and oral methods. It reduces the load that the oral route commonly places on the digestive tract and liver. It enhances patient compliance and minimizes harmful side effects of a drug caused from temporary overdose. Another advantage is convenience, especially notable in patches that require only once weekly application. Such a simple dosing regimen can aid in patient adherence to drug therapy. Designing and development of transdermal patches can be described as state of the art. The development of TDDS is multidisciplinary activity that encompasses fundamental feasibility studies starting from the selection of drug molecule to the demonstration of sufficient drug flux in an *ex vivo* and *in vivo* model followed by fabrication of a drug delivery system that meets all the stringent needs that are specific to the drug molecule (physicochemical and stability factors), the patient (comfort and cosmetic appeal), the manufacturer (scale up and manufacturability) and most important the economy[1].

The current transdermal delivery systems have evolved as a successful alternative to systemic drug delivery. Despite their relatively higher costs, transdermal delivery systems have proved advantageous for delivery of selected drugs, such as estrogens, testosterone, clonidine, nitroglycerin, scopolamine, fentanyl, and nicotine. Compared to oral dosage forms, these systems offer not only improved patient compliance, but also superior uniformity of drug concentrations in plasma throughout their duration of use. Most transdermal patches are designed to release the active ingredient at a zero-order rate for a period of several hours to days following application to the skin.[2]

Recently there has been an increasing awareness that the benefits of intravenous drug infusion can be closely duplicated, without its potential hazards, by continuous transdermal drug administration through skin[3.]

Acquired immune deficiency syndrome (AIDS), which threatens to cause a great plague in the present generation, was first identified in California in 1981. AIDS is considered to be an epidemic and according to estimates from the UN AIDS/WHO 2007 report on the global AIDS epidemic showed 33.2 million people lived with HIV, including 2.5 million children. An estimated 2.5 million (range 1.8-4.1 million) people were newly infected in 2007, including 420,000 children and it killed an estimated 2.1 million people, including 330,000 children. India has an estimated 2.5 million infections and an estimated adult prevalence of 0.36%. Life expectancy has fallen dramatically in the worst-affected countries; for example, in 2006 it was estimated that it had dropped from 65 to 35 years in Botswana. The annual number of AIDS deaths can be expected to increase for many years to come, unless more effective and patient compliant anti-retroviral medications are available at affordable prices. The major drawbacks of anti retroviral drugs for the treatment of AIDS are their adverse side effects during long-term therapy, poor patient compliance and huge cost of the therapy[4].

Lamivudine (3TC), an antiretroviral is commonly used in the treatment of HIV infected patients. Most of the antiretroviral including lamivudine are virustatic in nature and they must be administered for the life span of the patient (Bettyet al., 2000). Despite being quite effective on oral administration, 3TC exhibits dose dependent toxic side effects such as hepatotoxicity, hyperglycemia, hyperlipidemia, lactic acidosis, lipodystropy, osteonecrosis, osteoporosis, osteopenia, skin rashes, resulting from excessive systemic concentration. These side effects often require dosage reduction or even cessation of treatment, since conditions like lactic acidosis may even be fatal (Stephen et al., 1991 & Greenberg et al., 2004). Thus, despite their undisputed effectiveness several complicated clinical issues are associated with the use of these agents. Thus, the problems associated with oral administration of 3TC led us to explore the possibilities of designing novel drug delivery system for lamivudine with an alternative route of administration. Novel drug delivery carriers such as transdermal patches are very versatile to suit the delivery of various drug molecules (Joshi, 2000 & Mirachandani et al., 1993). To fulfill the need of long-term treatment with anti-HIV agents controlled drug delivery systems are preferred. Thus it was proposed that a noninvasive zero – order delivery such as the transdermal route is desirable,

as controlled delivery via oral route retains most of the drawbacks of conventional oral delivery. Percutaneous absorption of a number of antiretroviral has been studied indicating a promising future for this route for the delivery of antiretroviral (Bouwstra et al., 2002, Karali et al., 1995, Thomas et al., 2003)[5].

3.0 REVIEW OF LITERATURE

STRUCTURE OF SKIN

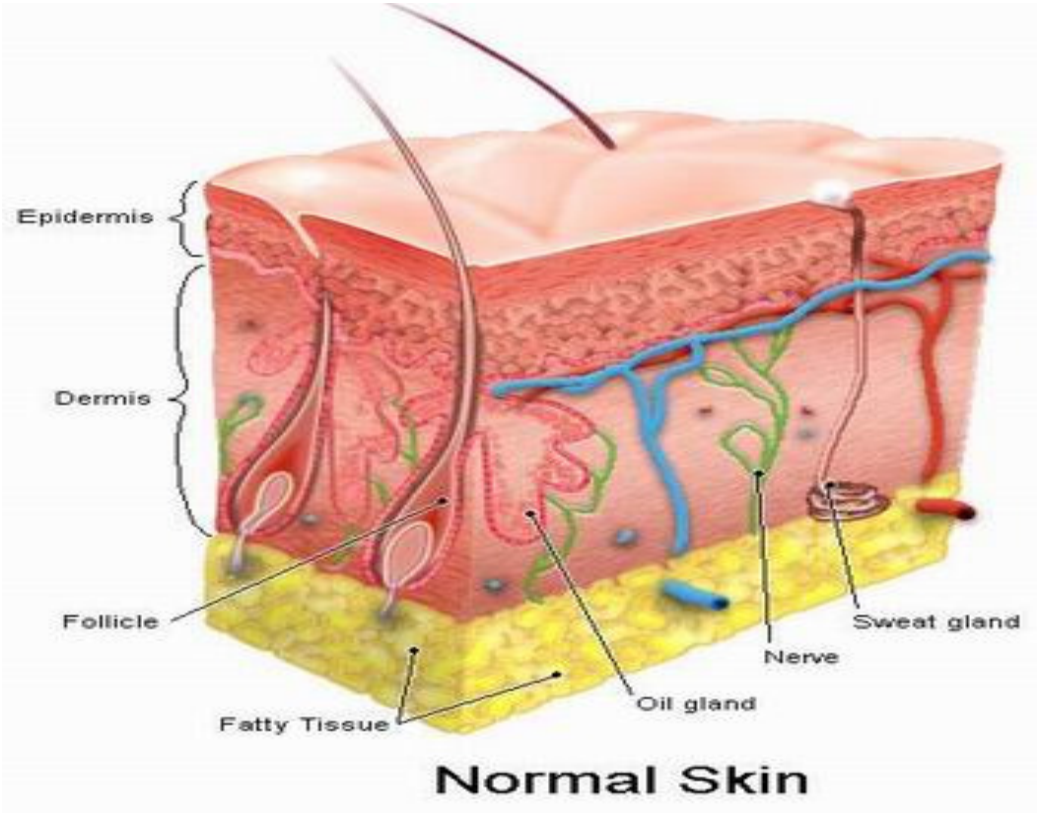


Fig.1 **Structure of skin**

The skin of an average adult body covers around $2m^2$ of the surface area and receives $1/3^{rd}$ of all blood circulating through the body. It has a thickness of only a fraction of mm, the skin separates underlying blood circulation network from the outside environment.

- An average human skin surface is known to contain, on the average, 40 to 70 hair follicles and 200 to 250 sweat ducts on each square cm of skin area.
- Presently there is an increasing recognition that skin can also serve as the part of administration for systemically active drugs.

- In this case, the drug applied topically will be absorbed first into blood circulation and then be transported to target tissues, which would be rather remote from the site of drug application to achieve its therapeutic doses.

The skin is a multi layer organ composed of many histological layers. It is generally described in terms of three major tissue organs.

1. The Epidermis
2. The Dermis
3. The hypodermis

The Epidermis

Epidermis is the most superficial layer and is composed of stratified squamous type of epithelium. From outside inward stratified epithelium may be divided in to 5 layers, they are,

a. Stratum Corneum

b. Stratum Lucidum

c. Stratum Granulosum

d. Stratum Spinosum

e. Stratum Germinatum

a. Stratum Corneum

The Stratum Corneum is most superficially placed and consists of many layers of compacted, flattened, dehydrated, and keratinized cells. They are dead cells converted into proteins and are continuously shed. The cell outlines are indistinct and the nuclei are absent. The stratum Corneum has a water content of only ~20% as compared to normal 70% in physiologically active Stratum Germinatum. This layer is thickest at the sole and the palm and thinnest at the lip. Hairs, loops, nails, feathers, scales, etc., are special outgrowth of this layer.

b. Stratum Lucidum

This is a thin more or less transparent layer 3 to 5 cells deep placed below the stratum corneum. The cell outlines are indistinct and the nuclei are absent. The cells contain droplets of "eleidin" which is precursor of keratin.

c. Stratum Granulosum

Stratum Granulosum is situated below the stratum Lucidum and consists of 3 to 5 layers of flattened polyhydral cells filled with keratohyalin granules which takes a deep stain with haematoxylin.

d. Stratum Spinosum

This is a broad layer of variable thickness and is made up of polyhedral cells is apparently covered with minute spines, which interdigitate with similar spines of adjacent cells. There are consequently known as "prickle cells". As the microscopic studies indicate that the prickle cells are in fact cytoplasmic protrusions and the branches from two cells actually do not have cytoplasmic continuity, but attached by well developed cytoplasmic nodes called as desmosomes.

e. Stratum Germinatum

This is growing layer is composed of a single layer of columnar epithelium which has got transverse, thin, short cytoplasmic processes on its basal lamina by means of which they anchor the epithelium to the underlying dermis. These cuboidal to columnar cells with oblong nuclei, placed perpendicularly on the basement membrane, produce new cells to replace those of the above layers by the process of mitosis.

The Dermis

The true skin is made up of connective tissue and lies below the epidermal layer which it supports and binds to the underlying tissues. It is made up chiefly of collagenous and elastic fibres which provide it with a tensile strength equal to that of a thin steel wire. This layer is utilized for the production of leather after chemical processing. From the structural point of view the superficial part of the dermis is compact and forms the papillary layer because it sends innumerable finger like projections into the prickle cell layer of epidermis. The deeper part of

the dermis is composed of rather loose connective tissue and is infiltrated with fat. The reticular layer of he dermis merges imperceptibly in to subcutaneous layer of fat.

Functions of skin

Protection

Stratum Corneum which is the outer most layer is horny and formed by the keratinized stratified epithelial cells resist the action of external agencies. It protects the internal individual injury and bacterial invasion. The nails are also defensive appendages of the skin.

Regulation of body temperature

Cutaneous vasoconstriction diverts the blood to the interior of the body and so diminishes heat loss. This is an important mechanism of protection against cold environment. Vasodilatation of the skin helps in elimination of heat from the body.

General sensation

The skin serves as the medium for receiving the general sensation. Tough, pain, temperature, etc., are subserved by the respective nerve endings present in the skin. The hair roots are richly supplied with nerves. Consequently, slight movement of the hair, such as by a blast of wind arouses. In this way hairs help the sensory functions of the skin.

Gaseous exchange

Absorption of oxygen and excretion of CO_2 may go on to a considerable extent through the skin in those animals whose skin is thin and moist, e.g. frogs. It is said that it can be carried to such an extent that these animals may live even after the extirpation of the lungs or in the hibernating period when the lungs do not function.

Absorption

Waxy layer hinders water absorption though the skin. But the skin is not completely waterproof and on prolonged exposure to water, there is water absorption causing swelling of the Stratum corneum. Lipids are easily permeable through the skin. Lipid-soluble substances like vitamins are easily absorbed through the skin.[6]

DRUG DELIVERY ROUTES ACROSS HUMAN SKIN

Drug molecules in contact with the skin surface can penetrate by three potential pathways: through the sweat ducts, *via* the hair follicles and sebaceous glands(collectively called the shunt or appendageal route), or directly across the stratum corneum (Fig. **1**). The relative importance of the shunt or appendageal route versus transport across the stratum corneum has been debated by scientists over the years (eg. [5-7]) and is further complicated by the lack of a suitable experimental model to permit separation of the three pathways. *In vitro* experiments tend to involve the use of hydrated skin or epidermal membranes so that appendages are closed by the swelling associated with hydration. Scheuplein and colleagues[7,8] proposed that a follicular shunt route was responsible for the presteady-state permeation of polar molecules and flux of large polar molecules or ions that have difficulty diffusing across the intact stratum corneum. However it is generally accepted that as the appendages comprise a fractional area for permeation of approximately 0.1% [9], their contribution to steady state flux of most drugs is minimal. This assumption has resulted in the majority of skin penetration enhancement techniques being focused on increasing transport across the stratum corneum rather than via the appendages. Exceptions are iontophoretic drug delivery which uses an electrical charge to drive molecules into the skin primarily via the shunt routes as they provide less electrical resistance, and vesicular delivery.

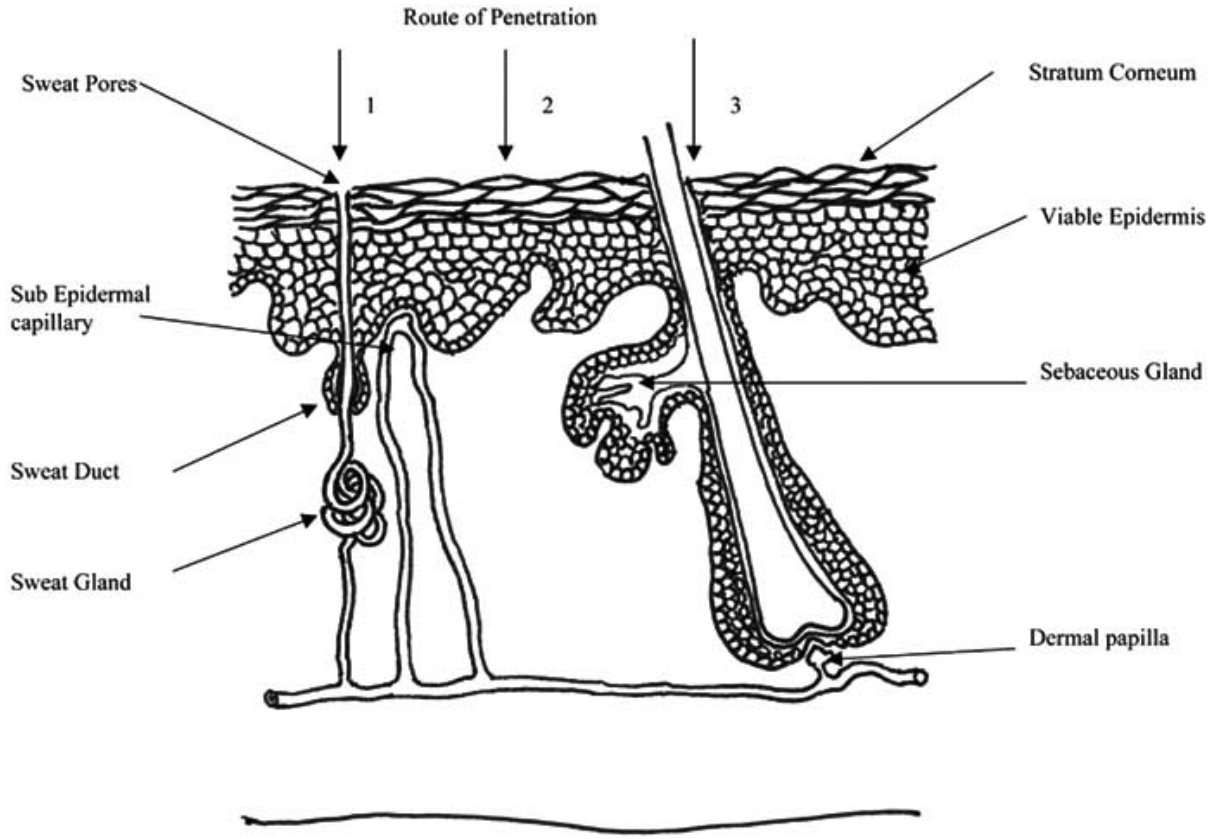


Fig: 2 **Simplified representation of skin showing routes of penetration: 1. through the sweat ducts; 2. directly across the stratum corneum;3. via the hair follicles**

Considerable research effort has been directed towards gaining a better understanding of the structure and barrier properties of the stratum corneum. A recent review by Menon provides a valuable resource[10]. The stratum corneum consists of 10-15 layers of corneocytes and varies in thickness from approximately 10-15 μm in the dry state to 40 μm when hydrated[11,12,13]. It comprises a multi-layered "brick and mortar" like structure of keratin-rich corneocytes(bricks) in an intercellular matrix (mortar) composed primarily of long chain ceramides, free fatty acids, triglycerides, cholesterol, cholesterol sulfate and sterol/waxesters[14]. However it is important to view this model in the context that the corneocytes are not brick shaped but are polygonal, elongated and flat (0.2-1.5 μm thick, 34-46 μm in diameter). The intercellular lipid matrix is generated by keratinocytes in the mid to upper part of the stratum granulosum discharging their lamellar contents into the intercellular space. In the initial layers of the stratum corneum this extruded material rearranges to form broad intercellular lipid lamellae[15], which then associate into lipid

bilayers[16,17], with the hydrocarbon chains aligned and polar head groups dissolved in an aqueous layer (Fig. 2). As a result of the stratum corneum lipid composition, the lipid phase behavior is different from that of other biological membranes. The hydrocarbon chains are arranged into regions of crystalline, lamellar gel and lamellar liquid crystal phases thereby creating various domains within the lipid bilayers[18]. The presence of intrinsic and extrinsic proteins, such as enzymes, may also affect the lamellar structure of the stratum corneum. Water is an essential component of the stratum corneum, which acts as a plasticizer to prevent cracking of the stratum corneum and is also involved in the generation of natural moisturizing factor (NMF), which helps to maintain suppleness.

In order to understand how the physicochemical properties of the diffusing drug and vehicle influence permeation across the stratum corneum and there by optimize delivery, it is essential to determine the predominant route of drug permeation within the stratum corneum. Traditionally it was thought that hydrophilic chemicals diffuse within the aqueous regions near the outer surface of intracellular keratin filaments (intracellular or transcellular route) whilst lipophilic chemicals diffuse through the lipid matrix between the filaments (intercellular route)[19] (Fig. 3).

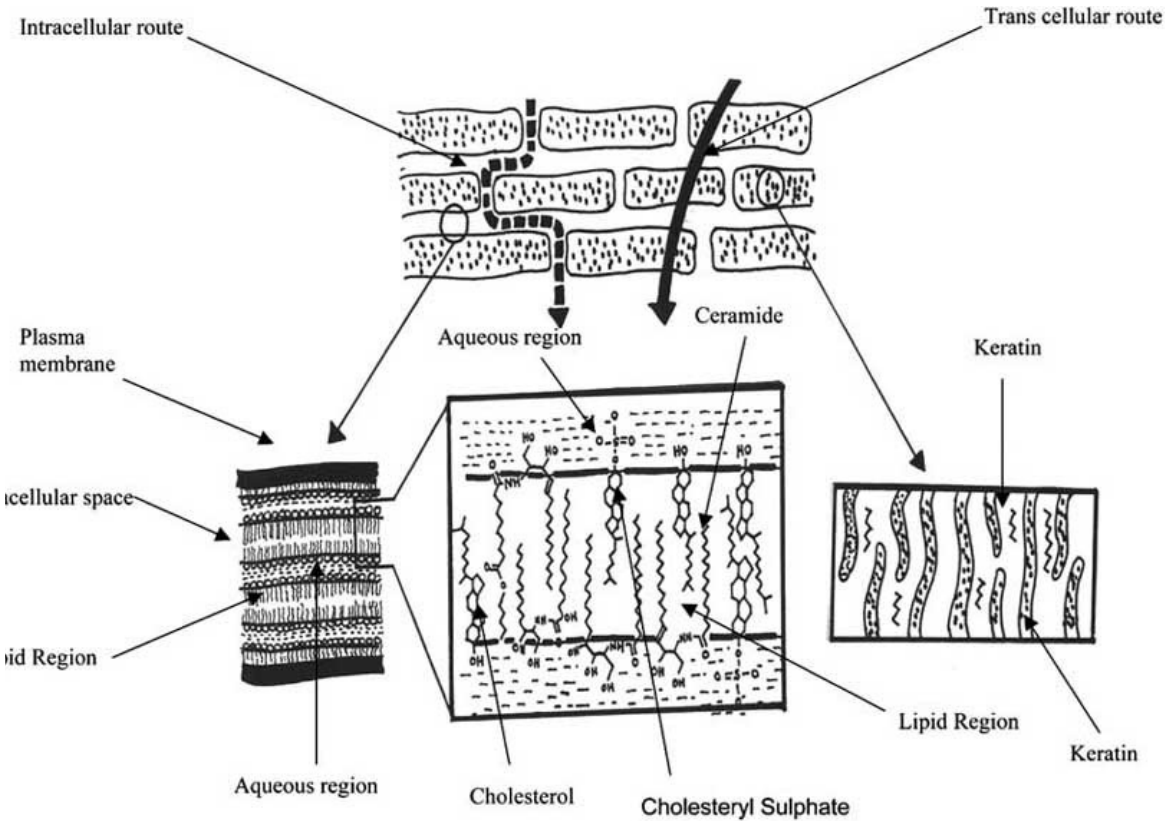


Fig: 3 **Diagrammatic representation of the stratum corneum and the intercellular and transcellular routes of penetration**[20]

However, this is an oversimplification of the situation as each route cannot be viewed in isolation. A molecule traversing via the transcellular route must partition into and diffuse through the keratinocyte, but in order to move to the next keratinocyte, the molecule must partition into and diffuse through the estimated 4-20 lipid lamellae between each keratinocyte. This series of partitioning into and diffusing across multiple hydrophilic and hydrophobic domains is unfavorable for most drugs. Consequently, based on more recent data (for example[15,21,22,23,24]) the intercellular route is now considered to be the major pathway for permeation of most drugs across the stratum corneum.

Percutaneous absorption

The drug molecules is of particular importance in the case of transdermal drug delivery systems because the drug has to be absorbed to an adequate extent and rate to achieve and maintain uniform, systemic, therapeutic levels throughout the duration of use. In general, once drug molecules cross the stratum corneal barrier, passage into deeper dermal layers and systemic uptake occurs relatively quickly and easily. Generally, drug absorption into the skin occurs by passive diffusion. The rate of drug transport across the stratum corneum follows Fick's Law of Diffusion[25.]

Fick's Law of Diffusion as applied to drug transport across stratum corneum[4]

$$\frac{dM}{dt} = \frac{D.\Delta C.K}{h}$$

where:

- dM/dt is the steady-state flux across stratum corneum
- D = is the diffusion coefficient or diffusivity of drug molecules
- ΔC = is the drug concentration gradient across the stratum corneum
- K = is the partition coefficient of the drug between skin and formulation medium, and
- h = is the thickness of the stratum corneum

KINETICS OF TRANSDERMAL PERMEATION

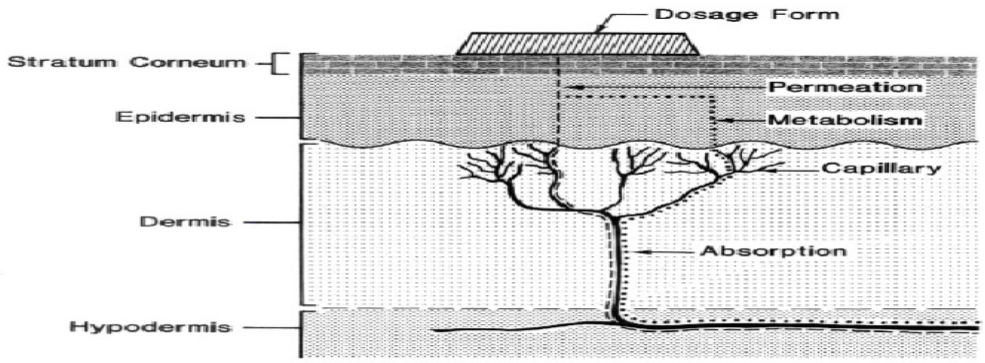


Fig: 4

Knowledge of skin permeation kinetics is vital to the successful development of transdermal therapeutic systems. Transdermal permeation of a drug involves the following steps:

1. Sorption by stratum corneum.

2. Penetration of drug through viable epidermis.

3. Uptake of the drug by the capillary network in the dermal papillary layer.

Thus permeation can be possible only if the drug possesses certain physiochemical properties.

The rate of permeation across the skin is given by:

$$dQ/dt = P_s (C_d - C_r) \quad .$$

Where C_d and C_r are the concentration of the skin penetrant in the donor compartment i.e. on the surface of stratum corneum and in the receptor compartment i.e. body respectively. Ps is the overall permeability coefficient of the skin tissue to the penetrant. This permeability coefficient is given by the relationship:

$$P_s = \frac{K_s D_{ss}}{h_s}$$

where K_s is the partition coefficient for the interfacial partitioning of the penetrant molecule from a solution medium or a transdermal therapeutic system on to the stratum corneum, D_{ss} is the apparent diffusivity for the steady state diffusion of the penetrant molecule through a thickness of skin tissues and h_s is the overall thickness of skin tissues. As K_s, Dss and h_s are constant under given conditions the permeability coefficient Ps for a skin penetrant can be considered to be constant. From equation (1) it is clear that a constant rate of drug permeation can be obtained only when $C_d >> C_r$ i.e. the drug concentration at the surface of the stratum corneum C_d is consistently and substantially greater than the drug concentration in the body C_r.

The equation becomes:

$$dQ/dt = P_s C_d$$

And the rate of skin permeation is constant provided the magnitude of C_d remains fairly constant throughout the course of skin permeation. For keeping C_d constant the drug should be released from the device at a rate R_r i.e. either constant or greater than the rate of skin uptake R_a, i.e $R_r >> R_a$.

Since $R_r >> R_a$, the drug concentration on the skin surface C_d is maintained at a level equal to or greater than the equilibrium solubility of the drug in the stratum corneum C_s. i.e. $C_d >> C_s$. Therefore a maximum rate of skin permeation is obtained and is given by the equation:

$$(dQ/dt)_m = P_s C_s$$

From the above equation it can be seen that the maximum rate of skin permeation depends upon the skin permeability coefficient P_s and is equilibrium solubility in the stratum corneum C_s. Thus skin permeation appears to be stratum corneum limited.

Kinetics of Drug Release From TDDS [26.]

Kinetics of release from monolithic systems[26]

In monolithic system the drug diffuses through the polymer and then partition into the skin from the system. Matrix diffusion occurs down the concentration gradient at a rate that is controlled by diffusion coefficient of drug molecular size of the drug. For the system, which release the drug by diffusion, were proposed by Higuchi. The steady state drug release from the matrix according to Higuchi eqution.

Where,

$$Q = \left[\frac{D\epsilon}{\tau} (2A - \epsilon C_s) C_s t \right]^{1/2} \quad \text{——————} \quad (1)$$

Q - Amount of drug release per unit area of the matrix exposed to the solvent.

A - Total concentration of drug in matrix.

D - Diffusion coefficient of drug in the permeation fluid.

ε - Porosity of the matrix.

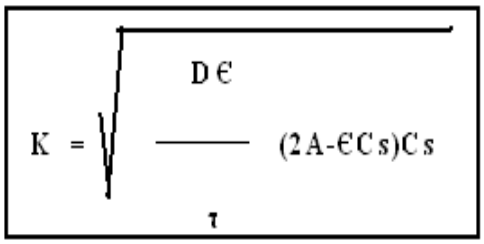
τ -Tortousity of the matrix.

Cs - Solubility of drug in dissolution medium.

t - Time.

It was assumed that A was greater that Cs by factor of at least 3 or 4 justifying the use of this particular equation. Assuming that the diffusion coefficient remain constant during release, then equation (1) may be reduced to

$$Q = K t^{1/2} \quad \text{——————} \quad (2)$$



Thus for diffusion controlled mechanism, a plot of percentage of drug release per unit area of the matrix against square root of time should be linear.The most important assumption in the theory of Higuchi is that the total surface areas of the matrix dose not change significantly during diffusion run. Two modes of behavior in such systems can be expected during diffusion studies,

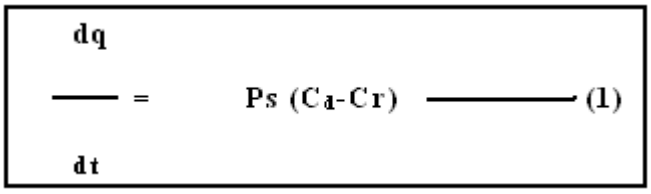
i. The drug will be released in first mode after an initial swelling of matrix.

ii In another mode, the drug may be diffused out without any swelling or change of geometry of the matrix.

Kinetic Release from membrane controlled systems[26]

In membrane controlled system, first the drug will partition from reservoir into polymer matrix that comprises the rate controlling membrane. In the membrane, diffusion will occur down a concentration gradient at a rate which will be controlled by the diffusion coefficient of the drug in the polymer. Once the drug has diffused through the rate controlling membrane it will partition into skin, diffusion occurs down the concentration gradient at a rate that is controlled by diffusion coefficient of drug in the polymer.

The rate of permeation dq/dt across various layer of skin tissue can be expressed as.



Where,

C_d - Concentration of drug in donor compartment.

Cr - Concentration of drug in receptor compartment.

Ps - Overall permeability coefficient.

Where as **Ps** can be defined as

$$P_s = \frac{K_{s/d}\, D_{ss}}{h_s} \qquad (2)$$

Where,

Ks/d- Partition coefficient.

Dss - Apparent diffusivity.

h_s - Thickness of the skin tissues.

Ps can be considered as constant, if Ks/d, Dss and hs terms in above equation are constant under a given set of conditions. Equation 1 suggest that to achieve a constant rate of drug permeation, one needs to maintain a condition in which the drug concentration of the surface of stratum corneum (Cd) is consistently and substantially greater than the drug concentration in the consistently and substantially greater than the drug concentration in the receptor side (Cr) i.e., Cd>>Cr under such condition equation1 can be reduced to,

$$\frac{dQ}{dt} = P_s C_d \qquad (3)$$

By making Cd greater than Cr, the drug concentration on the skin surface is maintained at level equal to or greater than equilibrium (or saturation) solubility of drug in stratum corneum Cs^e i.e., Cd $\geq Cs^e$ then equation 3 can be written as,

$$\left(\frac{dq}{dt}\right)_m = P_s C_s^e \qquad (4)$$

Penetration enhancers [27]

The perfect barrier properties of the epidermis restricts the transport through the skin to molecules with certain properties such as low molecular weight (< 500 Dalton), moderate lipophilicity (octanol–water partition coefficient between 10 and 1000), and modest melting point (< 200 °C) correlating with good solubility.

Dermatological and cosmetic preparations frequently contain active principles which can only act when they penetrate at least the outermost layer of the skin. However, the efficacy of topically applied actives is often suboptimal because the transport into the skin is slow due to the resistance of the outermost layer of the skin, the stratum corneum. Most small water-soluble non-electrolytes therefore diffuse into the systemic circulation a thousand times more rapidly when the horny layer is absent. Thus, a variety of means have been studied in attempts to overcome this barrier. Such strategies include physical, biochemical, and chemical methods.

Supersaturation

Supersaturation is a means to increase skin penetration without alteration of stratum corneum structure. The mechanism of enhancement is based simply on the increased Thermodynamic activity of the drug. This increases the concentration gradient (c0 – ci) in the Fick's law and thus forces the active principle out of the formulation and into and across the stratum corneum. Several methods can be used to produce supersaturated systems:

- Heating and subsequent cooling
- Removal of a solvent
- Reaction of two or more solutes to produce a compound which is less soluble
- Addition of a substance to a solution that reduces the solubility of the solute.

Water as penetration enhancer

Hydration of the stratum corneum is one of the primary measures to increase the penetration of most active compounds. Water opens up the compact structure of the horny layer. The water content of the horny layer can be increased either by delivering water from the vehicle to the skin or by preventing water loss from the skin when partially occlusive formulations are applied to the skin.

Chemical Enhancers

Several excipients are able to promote the transport of an active substance across the skin barrier by a variety of mechanisms. The most important are:

- Extraction of lipids from the stratum corneum
- Alteration of the vehicle/skin partitioning coefficient
- Disruption of the lipid bilayer structure
- Displacement of bound water
- Loosening of horny cells
- Delamination of stratum corneum

Chemical enhancers can be categorized into different groups. Solvents like alcohols, alkylmethyl sulfoxides, and polyols mainly increase solubility and improve partitioning coefficient. Moreover, some solvents, e.g. Dimethlysulphoxide (DMSO), ethanol, may extract lipids, making the stratum corneum more permeable. Oleic acid, Azone (epsilon-Laurocapram), and isopropyl myristate are typical examples of chemical enhancers which intercalate into the structured lipids of the horny layer where they disrupt the packing. This effect makes the regular structure more fluid and thus increases the diffusion coefficient of the permeant. Ionic surfactants, decylmethyl sulfoxide, DMSO, urea interact with the keratin structure in the corneocytes. This opens up the tight protein structure and leads to an increased diffusion coefficient D mainly for those substances which use the transcellular route.

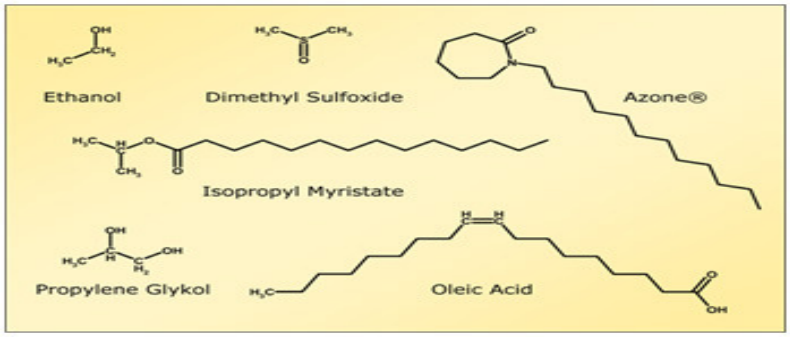


Fig: 5 **Different Examples of Chemical Enhancers**

An unfortunate feature of many potent chemical enhancers is that they irritate due to their ability to interact effectively with the corneocytes and the intercellular lipid structure.

Advantages of Transdermal patches

- Provide relatively steady and sustained drug concentration in plasma in contrast to conventional systems where peaks and troughs are a common feature.
- Variability due to factors such as pH intestinal motility, food intake, etc, which make vast difference in the bioavailability of the drugs given through oral route, are not existent.
- The hepatic first pass metabolism is avoided.
- A constant rate of absorption is possible in a vast variety of adverse patient population.
- Ease of administration and patient convenience.
- Drug input terminable by mere removal of the Transdermal patches.
- Drugs that cause gastro intestinal upset can be good candidates for Transdermal delivery because this method avoids direct effects on stomach and intestine.
- Increased therapeutic value due to avoidance of hepatic first pass effect, gastro intestinal irritation and low absorption problem.
- Drugs that are having short biological half-life can be given by this therapeutic systems and it also reduces dosing frequency.
- Transdermal patches are used for cessation of tobacco smoking.

Disadvantages of Transdermal patches

- Can be used only for drugs, which require very small plasma concentrations for action.
- Local irritation and arythmea are possible. Enzymes in epidermis or derived from micro organisms present on the skin may denature the drugs.
- Another significant disadvantage of Transdermal drug delivery is that skin is less permeable because it serves as protective barrier for the entry of foreign particles.
- In order to maintain constant release states, transdermal patches must contain surplus of active drug.

Preparation Of Different Types Of Transdermal Patches:

Several system designs have been used in development and fabrication of TDDSs. The systems that have been introduced in market can be classified into following types[28,29]:

Current controlled transdermal – release systems can be classified in to four categories:

1. Membrane permeation – controlled system in which the drug permeation is controlled by polymeric membranes.
2. Adhesive dispersion – type system in which drug is dispersed in to an adhesive polymer.
3. Matrix diffusion- controlled system in which the drug is homogeneously dispersed in a hydrophilic polymer.
4. Micro reservoir – controlled system in which microscopic spheres of drug reservoir are dispersed in a polymeric matrix.[3]
5. Drug in adhesive type- are directly in corporated into the organic solvent based pressure sensitive adhesive solution, mixed, cast as a thin film.

Matrix Type Transdermal Patch

Drug reservoir is prepared by dissolving the drug and polymer in a common solvent. The insoluble drug should be homogenously dispersed in hydrophilic or lipophillic polymer. The required quantity of plasticizer like dibutylpthalate, triethylcitrate, polyethylene glycol or propylene glycol and permeation enhancer is then added and mixed properly. The medicated polymer formed is then molded into rings with defined surface area and controlled thickness over the mercury on horizontal surface followed by solvent evaporation at an elevated temperature. The film formed is then separated from the rings, which is then mounted onto an occlusive base plate in a compartment fabricated from a drug impermeable backing. Adhesive polymer is then spread along the circumference of the film[30,31]. Commonly used polymers for matrix are cross linked polyethylene glycol, eudragits, ethyl cellulose, polyvinylpyrrolidone and hydroxypropylmethylcellulose.

The dispersion of drug particles in the polymer matrix can be accomplished by either homogenously mixing the finely ground drug particles with a liquid polymer or a highly viscous base polymer followed by cross linking of polymer chains or homogenously blending drug solids with a rubbery polymer at an elevated temperature[32]. The matrix system is exemplified by the development of Nitro-Dur®. Advantages of matrix patches include absence of dose dumping, direct exposure of polymeric matrix to the skin and no interference of adhesive. Design of matrix type patch is shown in Figure 6.

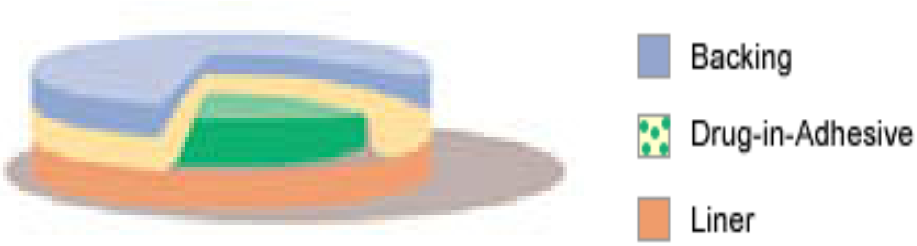


Fig: 6 **Matrix Type Transdermal Patch**

The rate of drug release from this type of system is defined as :

$$dQ/dt = \frac{AC_p D_p^{\frac{1}{2}}}{2t}$$

Where A is the initial drug loading dose dispersed in the polymer matrix and C_p and D_p are the solubility and diffusivity of the drug in the polymer respectively. Since, only the drug species dissolved in the polymer can release, C_p is essentially equal to C_R , where C_R is the drug concentration in the reservoir compartment.

Reservoir Type Transdermal Patch

The drug reservoir is made of a homogenous dispersion of drug particles suspended in an unleachable viscous liquid medium (e.g. silicon fluids) to form a paste like suspension or gel or a clear solution of drug in a releasable solvent (e. g. ethanol). The drug reservoir formed is sandwiched between a rate controlling membrane and backing laminate[33].

The rate controlling membrane can be nonporous so that the drug is released by diffusing directly through the material, or the material may contain fluid filled micropores in which case the drug may additionally diffuse through the fluid, thus filling the pores. In the case of nonporous membrane, the rate of passage of drug molecules depends on the solubility of the drug in the membrane and the thickness of membrane. Hence, the choice of membrane material is dependent on the type of drug being used. By varying the composition and thickness of the membrane, the dosage rate per unit area of the device can be controlled. Mostly EVA, ethyl cellulose, silicon rubber and polyurethanes are used to prepare rate controlling membranes[1,34,35,36]. EVA is used most frequently to prepare rate controlling membrane in transdermal delivery systems because it allows the membrane permeability to be altered by adjusting vinyl acetate content of polymer. Polyurethane membranes are suitable especially for hydrophobic polar compounds having low permeability through hydrophobic polymers such as silicon rubber or EVA membrane[37].

Liang *et al*., (1990) studied controlled release of scopolamine through EVA membrane in transdermal patch formulations and release rates were compared with uncontrolled reservoirs. It was found that an EVA membrane patch released scopolamine at a constant rate for more than 72 hours[35].

Krishna and Pandit (1994) prepared three transdermal formulations containing propranolol hydrochloride in a hydrophilic polymer matrix, one without rate controlling membrane and other two with EVA rate controlling membranes of different thickness. It was found that increased thickness of EVA led to greater retention of the drug in device and zero order profile was observed with EVA[36].

Rate controlling membrane may be prepared by solvent evaporation method or compression method. In case of solvent evaporation method, polymer is dissolved in solvent with or without plasticizer. Then the solution is poured on the horizontal surface and left for evaporation of solvent in order to obtain a thin film. In case of compression method, polymer is compressed with required force at high temperature for specific period of time[38]. Drugs that require relatively high doses or greater permeation enhancement, such as testosterone, use liquid reservoir systems. But the application of enhancers and adhesive technologies has allowed many drugs that were initially administered in liquid reservoirs to be used as matrix type systems e.g. estradiol, nicotine, nitroglycerine[39]. The main advantage of reservoir type patches is that this patch design can provide a true zero order release pattern to achieve a constant serum drug level. Examples of marketed preparations are Duragesic®, Estradem® and Androderm®. Figure 7 illustrates the design of reservoir type of patch.

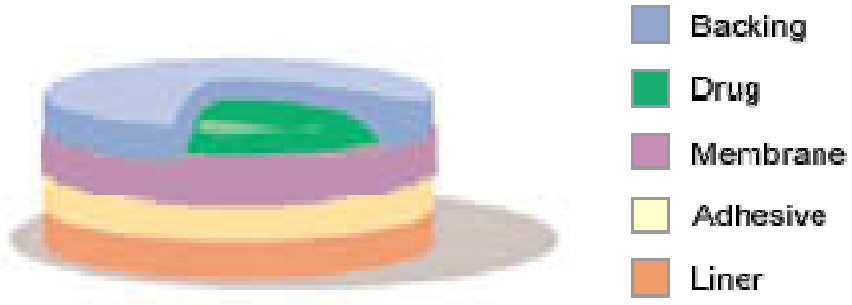


Fig: 7 **Reservoir Type Transdermal Patch**

The rate of drug release from this drug reservoir gradient controlled system is given by:

$$\frac{dQ}{dt} = \frac{K_{a/r} \cdot Da \times A(ha)}{ha(t)}$$

In the above equation, the thickness of the adhesive layer for drug molecules to diffuse through increases with time ha (t). To compensate for this time dependent increase in the diffusional path due to the depletion of drug dose by release, the drug loading level is also increased with the thickness of diffusional path A (ha).

Membrane matrix hybrid type patch

This is the modification of reservoir type transdermal patch. The liquid formulation of the drug reservoir is replaced with a solid polymer matrix (e.g. polyisobutylene) which is sandwiched between rate controlling membrane and backing laminate[40]. Examples of marketed preparations are Catapress® and TransdermScop®.

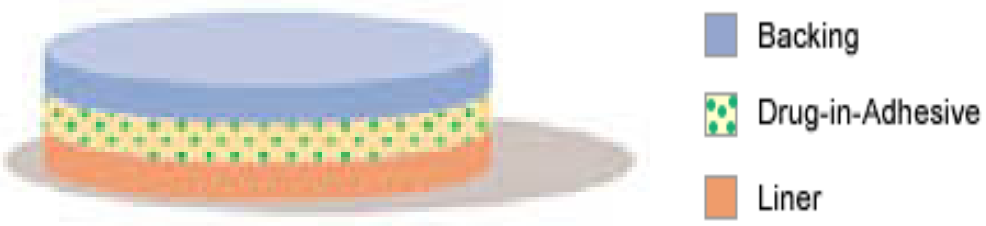


Fig: 8 **Membrane matrix hybrid type patch**

The intrinsic rate of drug release from this type of drug delivery system is defined by:

$$\frac{dQ}{dT} = \frac{Cr}{1/Pm + 1/Pa}$$

Where Cr is the drug concentration in the reservoir compartment and Pa and P m are the permeability coefficients of the adhesive layer and the rate controlling membrane , Pm is the sum of permeability coefficients simultaneous penetrations across the pores and the polymeric material. Pm and Pa, respectively, are defined as follows.

$$Pm = \frac{K_{m/r}.Dm}{hm}$$

$$Pa = \frac{K_{a/m}.Dm}{ha}$$

where $K_{m/r}$ and $K_{a/m}$ are the partition coefficients for the interfacial partitioning of drug from the reservoir to the membrane and from the membrane to adhesive respectively;

Dm and Da are the diffusion coefficients in the rate controlling membrane and adhesive layer, respectively; and hm and ha are the thickness of the rate controlling membrane and adhesive layer, respectively.

Micro reservoir type transdermal patch

The drug reservoir is formed by suspending the drug solids in an aqueous solution of water miscible drug solubilizer e.g. polyethylene glycol. The drug suspension is homogenously dispersed by a high shear mechanical force in lipophillic polymer, forming thousands of unleachable microscopic drug reservoirs (micro reservoirs). The dispersion is quickly stabilized by immediately cross linking the polymer chains in-situ which produces a medicated polymer disc of a specific area and fixed thickness. Occlusive base plate mounted between the medicated disc and adhesive form backing prevents the loss of drug through the backing membrane[41,42]. This system is exemplified by development of Nitrodisc®. Micro reservoir type transdermal system is shown in Figure 9.

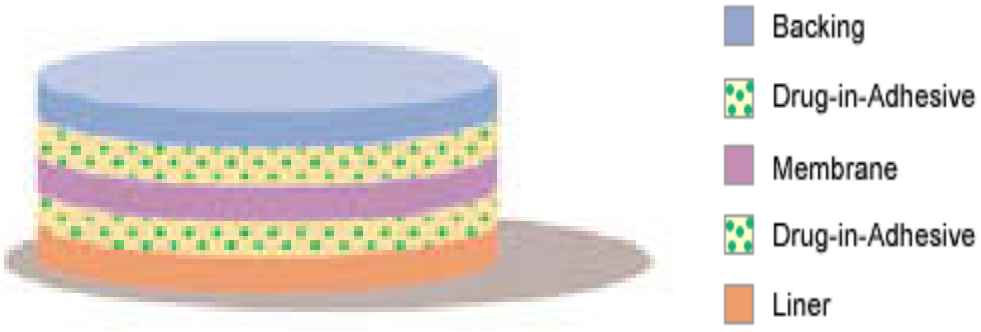


Fig: 9 **Micro reservoir type transdermal patch**

The rate of drug release in this system is defined by:

$$dQ/dt = \frac{K_{a/r} \cdot D_a}{ha} \times C_r$$

Where $K_{a/r}$ is the partition coefficient for the interfacial partitioning of the drug from the reservoir layer to adhesive layer.

Drug in adhesive type transdermal patch

The drug and other selected excipients, if any, are directly incorporated into the organic solvent based pressure sensitive adhesive solution, mixed, cast as a thin film and dried to evaporate the solvents, leaving a dried adhesive matrix film containing the drug and excipients. This drug in adhesive matrix is sandwiched between release liner and backing layer. Drug -in -adhesive patch may be single layer or multi layer. The multi layer system is different from single layer in that it adds another layer of drug-in-adhesive, usually separated by a membrane.

Some examples of suitable pressure sensitive adhesives are polysiloxanes, polyacrylates and polyisobutylene. These pressure sensitive adhesives are hydrophobic in nature and are prepared as solutions of polymer dissolved in organic solvents. Hence, this type of system is preferred for hydrophobic drugs as it is to be incorporated into organic solvent based hydrophobic adhesive[43]. Rachel *et al.,* (2004) prepared drug in adhesive patches of green tea extract and it was observed that major catechins and caffeine extracted from green tea were successfully delivered transdermally from drug-in-adhesive patches[44]. Kannikkanan *et al.,* (2004) prepared and evaluated monolithic drug in adhesive type transdermal patches of melatonin and used eudragit E100 as adhesive polymer[45]. Lake and Pinnock (2000) proved that once a week drug in adhesive patch of estrogen is more patient compliant as compared to twice a week reservoir patch. Characteristics of drug in adhesive patch may account for improved patient compliance due to ease of remembering once weekly patch application, improved cosmetic acceptance and better adhesion[46]. Examples of marketed preparations of drug-in-adhesives patches are Climara®, Nicotrol® and Deponit®. Design of this system is shown in Figure 10.

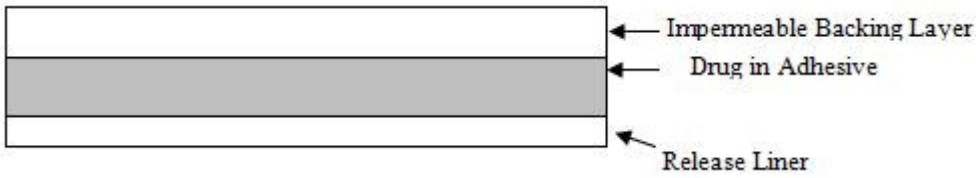


Fig: 10 **Drug in adhesive type transdermal patch**

Polymers for transdermal drug delivery systems[47]

Transdermal drug delivery technology represents one of the most rapidly advancing areas of novel drug delivery. This growth is catalyzed by developments in the field of polymer science.

Pharmaceutical polymers form an integral part of the transdermal drug delivery systems. Typically polymeric layers may be used as the protective outer covering to protect the dosage form from external damage. There may be a layer in which the drug is dispersed / dissolved within a polymeric matrix, which acts to control drug release to the skin. In addition, there is an adhesive layer, which is used to locate and maintain the dosage form at the site of application. Alternatively, in certain designs, the adhesive layer may additionally operate as the drug reservoir.

The major parts of TDS are a controlled release device composed of polymers, the drug, excipients and enhancers, a fastening system, usually a pressure-sensitive adhesive (PSA), to fix the device to the skin, and a hermetically sealed package composed of impervious film. Advances in transdermal drug delivery technology have been rapid because of the. Sophistication of polymer science which now allows incorporation of polymers in TDS in adequate quantity. The importance of polymer selection can be appreciated more if one considers the different design criteria which must be fulfilled

POLYMER USEFUL FOR TRANSDERMAL DEVICES[47]

Table-1

POLYMERS	ROLE
Natural polymers	
Gelatin	Base,adhesives
Sodium alginate	Base,adhesives
Gum Arabic	Base,adhesives
Starch	Base,adhesives
Gum tragacanth	Adhesives
Protein	Adhesives
Casein	Adhesives
Natural rubber	Base,adhesives
Semisynthetic polymers	
Cellulose acetate phthalate	
Methyl and ethyl cellulose	Base,adhesive
Nitrocellulose	
Hydroxy Propyl Cellulose	
Synthetic elastomers	
Polybutadiene	
Polyisoprene	Base with adhesive
Polysiloxane	
Silicone rubber	

Synthetic polymers	
Polyvinyl alcohol	Aqueous base,adhesive, baking membrane
Polypropylene	Linear,co-adhesive
Polyethylene	Co adhesive
polystyrene	Foam,backing
Polyurethane	Aq.base,adhesive
Polyvinyl pyrrolidone	linear
Polymethyl methacrylate	base
Polyvinyl acetate	linear
Polyhydroxy ethyl methcrylate	
Polyvinyl chloride	
polyacrylamide	base, adhesive
Polyethylene glycol	base,adhesive
Polyester	Base,adhesive
Polyamide polymer	Base,Linear,baking,foam
Epoxy ethyl vinyl acetate	Foam

Innovation in Transdermal Technology

The conventional passive means of applying drugs to skin include the use of vehicles such as ointments, creams, gels and patch technology. More recently, such dosage forms have been developed and/or modified in order to enhance the driving force of drug diffusion (thermodynamic activity) and/or increase the permeability of the skin. These approaches include the use of penetration enhancers, super saturated systems, hyaluronic acid, prodrugs, liposomes and other vesicles.

However, the amount of drug that can be delivered using these methods is still limited since the barrier properties of the skin are not fundamentally changed and as such, with the exception of patches, the majority are used to treat localized skin diseases where systemic absorption is not required. Thus, while new passive technologies typically offer an improvement in dose control, patient acceptance and compliance compared to more traditional semisolid formulations, they do not have the potential to widen the applicability of transdermal drug delivery unlike active transdermal drug delivery technologies.[48]

RECENT TECHNIQUES FOR ENHANCING TRANSDERMAL DELIVERY [49]:

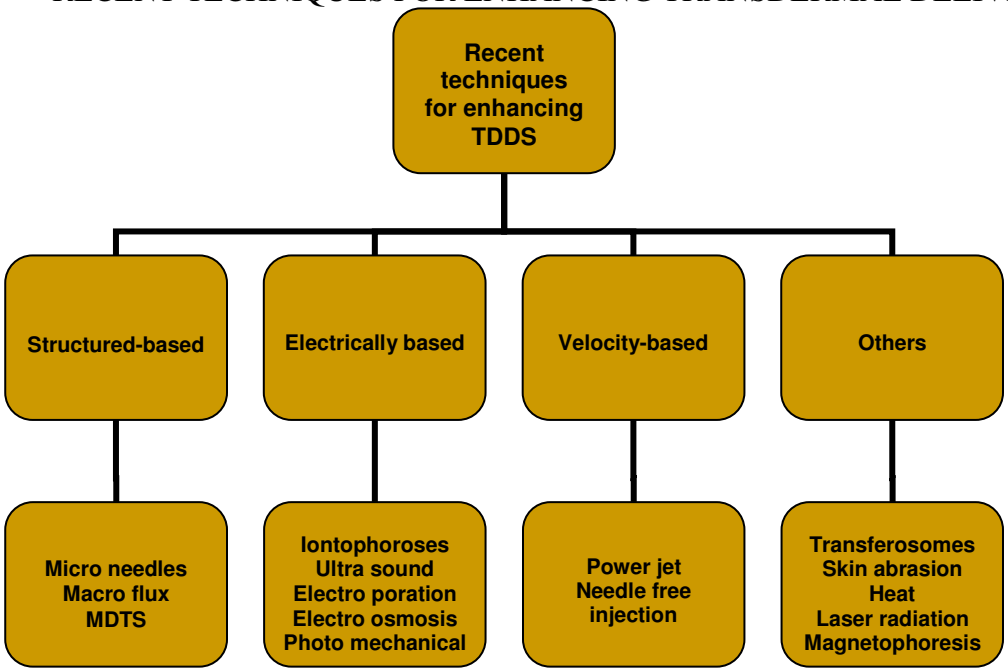


Fig- 11

However, the amount of drug that can be delivered using these methods is still limited. Since, the barrier properties of the skin are not fundamentally changed and as such with the exception of patches, the majority are used to treat localized skin diseases where systemic absorption is not required. Thus, while new passive technologies typically offer an improvement in dose control, patient acceptance and compliance compared to more traditional semisolid formulations, they do not have the potential to widen the applicability of transdermal drug delivery unlike active transdermal drug delivery technologies.

Active Methods

A rich area of research over the past 10 to 15 years has been focused on developing transdermal technologies that utilize mechanical energy to increase the drug flux across the skin by either altering the skin barrier (primarily the stratum corneum) or increasing the energy of the drug molecules. Recent progress in active transdermal drug delivery technologies has occurred as a result of advances in precision engineering (bio-engineering), computing, chemical engineering and material sciences, which have all helped to achieve the creation of miniature, powerful devices that can facilitate the generation of a required clinical response.

These so-called "active" transdermal technologies include iontophoresis, electroporation, microneedles, abrasion, needle-less injection, suction, stretching, ultrasound, magnetophoresis, radio frequency, lasers, photomechanical waves, and temperature manipulation. Some most commonly employed techniques include the following.

Iontophoresis

This method involves the application of a low level electric current either directly to the skin or indirectly via the dosage form in order to enhance permeation of a topically applied therapeutic agent. Products have already reached the US market using iontophoresis. e.g., recently, FDA approved a pre-filled, pre-programmed iontophoric device for sale in the United States. This product, called Lidosite^{TM,} delivers lidocaine and epinephrine to intact skin to provide local anesthesia for superficial dermatological procedures.

Electroporation

This method involves the application of high voltage pulses to the skin which has been suggested to induce the formation of transient pores. High voltages (Z100 V) and short treatment durations (milli seconds) are most frequently employed. Other electrical parameters that affect delivery include pulse properties such as waveform, rate and number. The technology has been successfully used to enhance the skin permeability of molecules

with differing lipophilicity and size (i.e. small molecules, proteins, peptides and oligonucleotides) including biopharmaceuticals with molecular weights greater that 7 KDA.

As electroporation improves the diffusion of such a wide range of compounds, it is thought that the pores created in the superficial layers of the skin are directly responsible for the increase in skin permeability. Genetronics, Inc. has developed a prototype electroporation transdermal device, which has been tested with various compounds with a view to achieving gene delivery, improving drug delivery and aiding the application of cosmetics.

Microneedle-based Devices

A new area of intense transdermal research and development is the development of devices that create micropores in the stratum corneum, the top most layer of the skin that serves as the greatest barrier to drug diffusion. Such devices include micro structured arrays, sometimes called microneedles, that when applied to the skin, painlessly create micropores in the stratum corneum without causing bleeding. These micropores offer lower resistance to drug diffusion than normal skin without micropores. The very first microneedle systems, described in 1976, consisted of a drug reservoir and a plurality of projections (microneedles 50 to 100 mm long) extending from the reservoir, which penetrated the stratum corneum and epidermis to deliver the drug. More recently, as a result of the rapid advancement in microfabrication technology in the last 10 years, numerous cost-effective methods of producing microneedle devices have been developed. The ALZA Corp. has recently commercialized a microneedle technology named Macroflux which can either be used in combination with a drug reservoir or by dry coating the drug on the microprojection array; the latter being better for intracutaneous immunization.

Skin Abrasion

The abrasion technique involves the direct removal or disruption of the upper layers of the skin to facilitate the permeation of topically applied medicaments. Some of these devices are based on techniques employed by dermatologists for superficial skin resurfacing

(e.g. microdermabrasion) which are used in the treatment of acne, scars, hyperpigmentaion and other skin blemishes.

Microscissuining is a process which creates micro channels in the skin by eroding the impermeable outer layers with sharp microscopic metal granules. Carlisle Scientific is currently in the process of developing a pen-like handheld device called the microscissioner.

In addition, Med Pharm Ltd. has recently developed a novel dermal abrasion device (D3S) for the delivery of difficult to formulate therapeutics ranging from hydrophilic low molecular weight compounds to biopharmaceuticals. *In vitro* data has shown that the application of the device can increase the penetration of angiotensin into the skin 100-fold compared to untreated human skin. This device is non-invasive and histological studies on human skin show that the effects on the stratum corneum are reversible and non-irritating.

Needle-less Injection

This is reported to involve a pain-free method of administering drugs to the skin. Over the years, there have been numerous examples of both liquid (Ped-O-Jet, Iject, Biojector2000, Medi-jector and Intraject) and powder (PMED device formerly known as Powderject injector) systems. The latter device has been reported to successfully deliver testosterone, lidocaine hydrochloride and macromolecules such as calcitonin and insulin.

This method of administering drugs circumvents issues of safety, fear and pain associated with the use of hypodermic needles. Transdermal delivery is achieved by firing the liquid or solid particles at supersonic speeds through the outer layers of the skin using a suitable energy source. The PMED device consists of a Helium gas cylinder, drug powder sealed in a cassette made of plastic membrane, a specially designed convergent-divergent supersonic nozzle and a silencer to reduce the noise associated with the rupturing of the membrane when particles are fired.

The mechanism involves forcing compressed gas (Helium) through the nozzle, with the resultant drug particles entrained within the jet flow reportedly traveling at sufficient velocity for skin penetration. An essential difference between administration of a DNA

vaccine by needle injection or by PMED is the efficiency with which the administered DNA generates the encoded protein for presentation on the surface of antigen-presenting cells (APCs). Using PMED, it is possible to deliver the DNA directly to the intracellular compartment of cells within the epidermis, and because the epidermis is rich in APCs, significant numbers can potentially be targeted with each administration. This is supported by non-clinical studies in pigs that have included histological examination of PMED administration sites.

Ultrasound (sonophoresis and phonophoresis)

This technique involves the use of ultrasonic energy to enhance the transdermal delivery of solutes either simultaneously or via pre-treatment and is frequently referred to as sonophoresis or phonophoresis. The SonoPrep device (Sontra Medical Corp.) uses low frequency ultrasound (55 KHz) for an average duration of 15 seconds to enhance skin permeability. This battery-operated, handheld device consists of a control unit, ultrasonic horn with control panel, a disposable coupling medium cartridge, and a return electrode.

Laser Radiation

This method involves direct and controlled exposure of a laser to the skin which results in the ablation of the stratum corneum without significantly damaging the underlying epidermis. Removal of the stratum corneum using this method has been shown to enhance the delivery of lipophilic and hydrophilic drugs. A handheld portable laser device has been developed by Norwood Abbey Ltd. (Victoria, Australia), which in a study involving human volunteers, was found to reduce the onset of action of lidocaine to 3 to 5 minutes, while 60 minutes was required to attain a similar effect in the control group. The Norwood Abbey system has been approved by the U.S. and Australian regulatory bodies for the administration of a topically-applied anaesthetic. Laser systems are also being developed to ablate the stratum corneum from the epidermal layer. As with microneedles, the ablated regions offer lower resistance to drug diffusion than non-ablated skin. One company has recently received FDA approval to market this device with a lidocaine cream Dispenser for Transdermal Patches.

Magnetophoresis, which is still in the research phase, enhances skin permeability by applying a magnetic field.

TRANSDERMAL MARKET

The market for transdermal products has been in a significant upward trend that is likely to continue for the foreseeable future. An increasing number of TDD products continue to deliver real therapeutic benefit to patients around the world. More than 35 TDD products have now been approved for sale in the US.

Table -2

Product name	Drug	Manufacturer	Indication
Alora	Estradiol	TheraTech/Proctol and Gamble	Postmenstrual syndrome
Androderm	Testosterone	TheraTech/GlaxoSmithKline	Hypogonadism in males
Catapres-TTS	Clonidine	Alza/Boehinger Ingelheim	Hypertension
Climaderm	Estradiol	Ethical Holdings/Wyeth-Ayerest	Postmenstrual syndrome
Climara	Estradiol	3M Pharmaceuticals/Berlex Labs	Postmenstrual syndrome
CombiPatch	Estradiol/Norethindrone	Noven , Inc./Aventis	Hormone replacement therapy
Deponit	Nitroglycerin	Schwarz-Pharma	Angina pectoris
Duragesic	Fentanyl	Alza/Janssen Pharmaceutica	Moderate/severe pain
Estraderm	Estradiol	Alza/Norvatis	Postmenstrual syndrome
Fematrix	Estrogen	Ethical Holdings/Solvay Healthcare	Postmenstrual syndrome
FemPatch	Estradiol	Parke-Davis	Postmenstrual syndrome
Habitraol	Nicotine	Novartis	Smoking cessation
Minitran	Nitroglycerin	3M Pharmaceuticals	Angina pectoris
Nicoderm	Nicotine	Alza/GlaxoSmithKline	Smoking cessation
Nicotrol	Nicotine	Cygnus Inc./McNeil Consumer Products, Ltd.	Smoking cessation
Nitrodisc	Nitroglycerin	Roberts Pharmaceuticals	Angina pectoris
Nitro-dur	Nitroglycerin	Key Pharmaceuticals	Angina pectoris
Nuvelle TS	Estrogen/Progesterone	Ethical Holdings/Schering	Hormone replacement therapy
Ortho-Evra	Norelgestromin/estradiol	Ortho-McNeil Pharmaceuticals	Birth control
Prostep	Nicotine	Elan Corp./Lederle Labs	Smoking cessation
Testoderm TTS	Testosterone	Alza	Hypogonadism in males
Transderm Scop	Scopolamine	Alza/Norvatis	Motion sickness

The pie diagram given below shows that Fentanyl and nitroglycerine are the drugs most popularly marketed using transdermal patches.

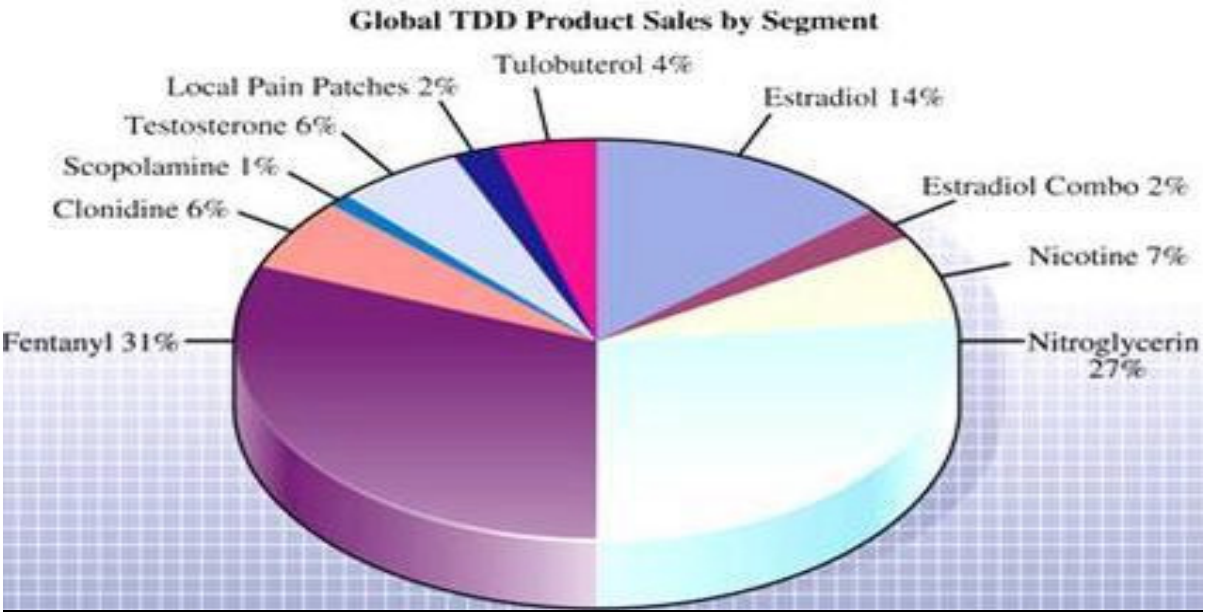


Fig-12

Table-3: **US Sales of Select Prescription Transdermal Product**

Drug	Examples of Brand Names	Annual Sales (US$) MAT 9/00	Annual Sales (US$) MAT 9/01	Annual Sales (US$) MAT 9/02
Fentanyl	Duragesic	1.15b	1.29b	1.59b
Estradiol	Climara, Vivelle-Dot, CombiPatch	260m	253m	279m
Clonidine	Catapres TTS	133m	147m	168m
Nitroglycerin	Nitro-Dur, Deponit	209m	182m	159m
Nicotine	Nicoderm CQ, Nicotrol	88m	72m	73m
Testosterone	Testoderm TTS	43m	44m	50m
Ethinylestradiol and Norelgestromin	Ortho Evra			19m
Scopolamine	Transderm-Scop	12m	14m	17m
Lidocaine	Lidoderm		27m	60m
Oxybutynin	Awaiting FDA approval			
Methylphenidate	Awaiting FDA approval			

Table-4**: Over-the-Counter Patch Products Currently Marketed in US**[50]

NeoSkin Aromatherapy Cucumber Pads	*HerbalPad Echinacea and Golden Seal*
NeoSkin Pre-formed Moisture Mask	*HerbalPad Ginkgo Biloba*
NeoSkin Hydrating Wrinkle Patch	*HerbalPad Glucosamine and Chondroitin*
NeoSkin Pre-formed Moisture Mask	*HerbalPad St. John s Wort*
DuraPatch	*Excel Creatine Patch*
ThermaCare Heatwraps	*HoMedics Magnetic Therapy Patch*
IcyHot Patch	*Neutrogena On-the-Spot Acne Patch*
Excedrin Cooling Patches	*SunSpots Patches*
TheraPatch Cold Sore	*Curad Scar Therapy Patches*
TheraPatch Psoriasis	*Dr. Scholl s Clear Away Wart Removal*
TheraPatch Vapor Kids	*Nicotine Patches (various brands)*
TheraPatch Anti-Itch	*Crest Whitestrips*

The Future of TDDS - New Systems

Several exciting 'active' transdermal drug delivery systems are also on the horizon. Systems using external stimuli to drive the drug into the skin will offer rate-controlled, on-demand delivery of those drugs with a larger molecular weight which until now were not deliverable by passive transdermal patches. For example, iontophoresis uses a miniature battery to establish an electrical potential between the adhesive and the skin in a TDD patch, which uses a conductive adhesive.

A mild electrical current delivers an ionically charged drug into the skin. Currently, **reverse iontophoresis** has been developed for use as a diagnostic tool in the medical diagnostics industry for blood glucose monitoring, attracting fluid out of the skin that can be analysed. **Sonophoresis**, which uses ultrasound waves, is also being tested. A portable device emits sound waves through a patch attached to the device for painless delivery of a drug through the skin. The adhesive used must be able to withstand the effects of the sound waves.

Yet another method is **electroporation**, which uses electric current to change the surface properties of the skin, creating channels of low transmission-resistance and thereby accelerating drug delivery.

Further, **buccal or transmucosal patches** have been designed to be placed in the mouth to deliver a drug through the mucous membranes. This technique will allow much higher drug flux and enable large, higher molecular weight drugs to be administered transdermally. The challenge for adhesive manufacturers is to develop an adhesive that adheres to wet surfaces on the interior of the mouth and will not dissolve in the presence of an aqueous environment.

In the future, we can expect advances in passive transdermal patches to include:

- Extended wear patches with stronger cohesion properties to remain at a fixed point on the skin without movement
- Bi-phasic drug delivery profiles such as time-delayed or time moderated delivery
- Smaller, more aesthetically acceptable patches with increased solubility of the drug in the adhesive for a higher diffusion rate
- Generic drug patches
- Combinational drug patches, such as nicotine with an anti-irritant that delivers more than one drug, each with a different size molecule and potentially different therapeutic levels.

The adhesive manufacturers who are succeeding in the TDDS market are those that have invested in partnerships with pharmaceutical companies. These companies require the adhesive manufacturers to be deeply involved in the product development process, conducting research, testing and materials qualification for adhesive formulation and ultimately.

4.0 REVIEW OF LAMIVUDINE

Formulation and Evaluation of Ethosomes for Transdermal Delivery of Lamivudine to investigate the mechanism for improved intercellular and intracellular drug delivery from ethosomes using visualization techniques and cell line study, **and concluded** that the visualization study indicate that ethosomal formulation affected the normal histology of skin by producing lipid perturbation and increasing the intercellular lipid lamellae space of the SC[51].

Preparation and characterization of Lamivudine microcapsules using various cellulose polymers the study was to prepare and evaluate microcapsules for the controlled release of lamivudine using various cellulose polymers **and concluded** that the release kinetics data and characterization studies indicate that drug release from microcapsules was diffusion – controlled and that the microcapsules were stable[52].

Design and Study of Lamivudine Oral Controlled Release Tablets the study was to design oral controlled release matrix tablets of lamivudine using hydroxypropyl methylcellulose (HPMC) as the retardant polymer and to study the effect of various formulation factors such as polymer proportion, polymer viscosity, and compression force on the *in vitro* release of drug, **and concluded** that the CR matrix tablets of LAM conforming to good quality were prepared using HPMC by the wet granulation method. Release rate of the drug from the matrix tablets was dependent on proportion as well as viscosity of HPMC used, which release 20%to 30%of drug in the first hour and extend the release up to 16 to 20 hours, can overcome the disadvantages associated with conventional tablet formulations of LAM[53].

Lamivudine Liposomes for Transdermal Delivery - Formulation, Characterization, Stability and *In vitro* Evaluation to study the preparation of liposomes of Lamivudine by thin film hydration and their potential for transdermal drug delivery has been evaluated *in vitro* ,**and concluded** that formulated 3TC liposomes were stable and have shown appreciably controlled skin permeation as well as minimal retention

of drug molecules in the skin. Also by achieving controlled release of lamivudine across the skin, it is possible to achieve a reduction in dose, while maintaining the required drug concentration[5].

Dissolution test for lamivudine tablets: Optimization and statistical analysis to study the comparison of different methods for dissolution test used by five different manufacturer laboratories of lamivudine tablets is made, evaluated, and discussed…**and concluded** the methods and conditions established by the manufacturer laboratories, all batches presented equivalent results to the reference product. However, when the method and criteria proposed by this work were used, smaller DR% values were observed for batches C1, C2, and C3 when compared to those of reference, and therefore could not be considered equivalent[54].

Preparation, Evaluation and *in vitro – in vivo Correlation (IVIVC)* study of Lamivudine Loaded Microspheres to study the establishment of an *Invitro-Invivo correlation* of prepared sustained release microspheres and compared with conventional Lamivudine tablet after administrating orally to New Zealand white rabbit species..**and concluded** that all the formulated microspheres showed identical pharmacological effect in comparison to standard lamivudine tablet. Parameters like dissolved fraction absorbed, DT vs. MRT and $T_{85\%}$ revealed a significant *in vitro- in vivo correlation*[55].

5.0 DRUG PROFILE

LAMIVUDINE[56,57,58]

Chemical Name **:** (2R, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidine - 2-one.

Molecular Formula **:** $C_8H_{11}N_3O_3S$

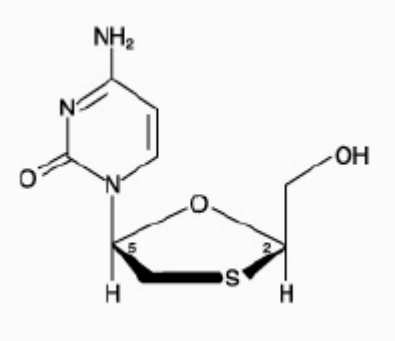
Molecular Weight **:** 229.3

Physical properties **:** white to off-white crystalline solid.

Solubility **:** Freely soluble in water.

Melting point : 160-162^{0}c

Structure **:**



Mechanism of action

Lamivudine is a selective inhibitor of HIV-1 and HIV-2 replication, including zidovudine-resistant clinical isolates of the human immunodeficiency virus (HIV). Lamivudine is a synthetic nucleoside analogue. Intracellularly, lamivudine is phosphorylated to its active 5′-triphosphate metabolite, lamivudine triphosphate, 3TC-TP. Incorporation of the monophosphate form into viral DNA by HBV reverse transcriptase results in DNA chain termination. 3TC-TP also inhibits the RNA- and DNA-dependent DNA polymerase

activities of HIV-1 reverse transcriptase (RT). 3TC-TP is a weak inhibitor of mammalian α, β, and γ-DNA polymerases.

Clinical Uses

Lamivudine is indicated for the treatment of HIV when used in combination with other antiretroviral agents.A lower dose than that used to treat HIV is approved for the treatment of HBV. Although lamivudine initially improves histological and biochemical measures of hepatic function and reduces HBV DNA to below the limits of detection, withdrawal of the drug usually results in disease recurrence. Resistance appears in up to one third of patients after 1 year of treatment.

Pharmacokinetics

Adults

Onset of action	**:**	Prompt
Time to peak, serum	**:**	1 hour
Oral Bioavailability	**:**	80%
Half life elimination	**:**	5-7 hours (oral)
Excretion	**:**	Urine.
Protein binding	**:**	less than 36%
Renal excretion	**:**	70%
Volume of distribution	**:**	1.3 L/kg
Plasma concentration	**:**	1.4 µg/ml

Drug interactions

Lamivudine and zalcitabine may inhibit the intracellular phosphorylation of one another. Therefore, use of lamivudine in combination with zalcitabine is not recommended.

Pregnancy : No evidence of teratogenicity due to lamivudine was observed **.**

Nursing Mothers: Lamivudine is also excreted in human milk. Samples of breast milk obtained from 20 mothers receiving lamivudine monotherapy (300 mg twice daily) or combination therapy (150 mg lamivudine twice daily and 300 mg zidovudine twice daily) had measurable concentrations of lamivudine. Because of the potential for serious adverse reactions in nursing infants, mothers should be instructed not to breastfeed if they are receiving lamivudine.

ADVERSE REACTIONS

Non-site Specific **:** Malaise and fatigue, Fever or chills

Ear, Nose, and Throat : Ear, nose and throat infections, Sore throat

Gastrointestinal **:** Nausea and vomiting, abdominal discomfort and pain,

Musculoskeletal **:** Myalgia, Arthralgia, Neurological, Headache

Paediatric Patients with HIV Infection: In early open-label studies of lamivudine in children with HIV, peripheral neuropathy and neutropenia were reported, and pancreatitis was observed in patients.

DOSAGE AND ADMINISTRATION

Adults: The recommended oral dose of lamivudine is 150mg (twice daily) for treatment of HIV in adults.

Pediatric Patients: The recommended oral dose of lamivudine for pediatric patients 2 to 17 years of age with HIV is 3 mg/kg once daily up to a maximum daily dose of 100 mg.

6.0 POLYMER PROFILE

HYDROXY PROPLY METHYL CELLULOSE [59,60]

It is mixed alkyl-hydroxy alkyl cellulose ether and may be regarded as the propylene glycol ether of methyl cellulose.

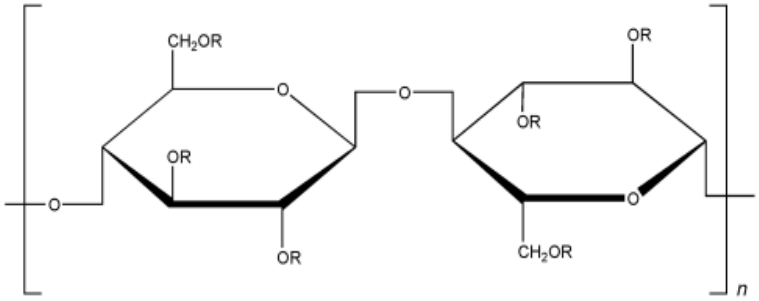
BP : Hypromellose

USP : Hydroxy propyl methyl cellulose

Chemical name : Cellulose, 2- hydroxypropyl methylether.

Empirical formulae : Hydroxy propyl methyl cellulose is partly O-methylated and O-(2-hydroxypropylated) cellulose. It is available in several Grades which vary in viscosity and extend of substitution

Structure



where R is H, CH_3, or $CH_3CH(OH)CH_2$

Molecular weight : 4000 mol. daltons

SYNONYM : HYPROMELLOSE

Trade name : Methocel

Solubility	**:**	Methocel soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol 95% and ether, but soluble in mixtures of ethanol and dichloro methane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol.
Description	**:**	An odorless and tasteless, white or creamy-white fibrous(or) granular powder.
Density	**:**	Bulk : 0.341 g/cm^3
	:	Tapped : 0.557 g/cm^3
	:	True : 1.326 g/cm^3
Specific gravity	**:**	1.26 g/cm^3
Viscosity	**:**	Low viscosity grade
Grade	**:**	HPMC K 4M
pH	**:**	5.5–8.0 for a 1% w/w aqueous solution.
Stability	**:**	Very stable in dry conditions. Solutions are stable at pH 3-11
Functional category	**:**	Coating agent; film former; rate controlling polymer for Sustained release; stabilizing agent; viscosity-increasing agent; tablet binder.
Applications	**:**	1) In oral products, hypromellose is primarily used as a tablet Binder, in film-coating, and as a matrix for use in extended release tablet formulations. 2) It is widely used in oral and topical pharmaceutical formulation.

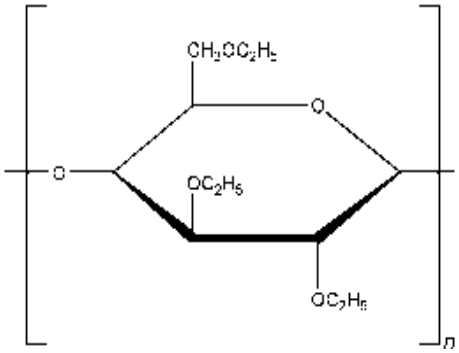
3) Concentrations between 2% and 5% w/w may be used as abinder in either wet- or dry-granulation processes.

4) High-viscosity grades may be used to retard the release of drugs from a matrix at levels of 10–80% w/w in tablets and capsules.

ETHYL CELLULOSE[61]

Chemical name : Cellulose ethyl ether.

Structure :



Functional Category : Coating agent; flavoring fixative; tablet binder; tablet filler; Viscosity - increasing agent.

Solubility : Soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and toluene. Practically insoluble in glycerin, propylene glycol, and water.

Description : Ethylcellulose is a tasteless, free-flowing, white to light tan colored powder.

Bulk density : 0.4 g/cm^3

Viscosity : 7 to 100 mPa s for a 5% w/v solutions

Specific gravity : 1.12–1.15 g/cm^3

Stability	**:**	Ethylcellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters.
Incompatibility	**:**	Incompatible with paraffin wax and microcrystalline wax.
Safety	**:**	Ethylcellulose is widely used in oral and topical Pharmaceutical formulations. It is also used in food products.
Application	**:**	1) The main use of ethylcellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. 2) Ethylcellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation; for example, where granules are coated with ethylcellulose to inhibit oxidation. 3) Modified-release tablet formulations may also be produced using ethylcellulose as a matrix former.

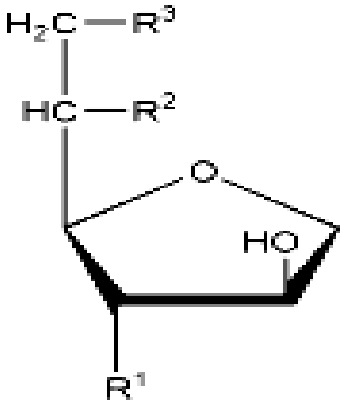
SPAN-80

Synonyms: Sorbitan Monolaurate

Empirical Formula: $C_{18}H_{34}O_6$

Molecular Weight: 346

Structural formula:



$R^1 = R^2 = OH$, $R^3 = R$ for sorbitan monoesters

where R = $(C_{11}H_{23})COO$ for laurate

Functional category: Emulsifying agent; nonionic surfactant; solubilizing agent; wetting and dispersing/suspending agent.

Application in pharmaceutical formulation

- Span 80 are widely used in cosmetic, food products, and pharmaceutical formulations as lipophilic non-ionic surfactants.
- They are mainly used in pharmaceutical formulations as emulsifying agent in the preparation of creams, emulsion, and ointments for topical application.
- When used alone span 80 produce stable water –in-oil emulsion and micro emulsion.

Description: Sorbitan esters occur as cream to amber-colored liquids or solid with a distinctive odor and taste. Span 80 occurs as yellow viscous liquid.

Typical properties

Table- 5

Permeation enhancer	**Density (g/cm^3)**	**HLB value**	**Viscosity(mPs)**
Span 80	1.01	8.6	2970-4080

Stability and storage condition

Gradual soap formation occurs with strong acid or bases; sorbitan esters are stable in weak acid or bases. Sorbitan esters should be stored in a well- closed container in a cool, dry place.

Safety

They are generally regarded as non-toxic and non-irritant materials. However, there have been occasional reports of hypersensitive skin reaction following the topical application of product containing sorbitan esters. When heated to decomposition the sorbitan esters emit acrid smoke and irritating fumes.

7.0 OBJECTIVE OF THE STUDY

Study has been carried to provide an Anti-viral drug in transdermal delivery system. The main object is to evaluate the feasibility of sustained controlled delivery of therapeutically effective amount of nucleoside reverse transcriptase inhibitors in Matrix type transdermal delivery system.

The nucleoside reverse transcriptase inhibitors have become the first-line therapy in treating HIV and HBV patients..

The transdermal drug delivery system has gained popularity over the past few decades. The major penetration pathway of drug molecules through the stratum corneum of intact human skin is by diffusing through the lipid envelopes of the skin cells.

Lamivudine was selected among the nucleoside reverse transcriptase inhibitors due to the molecular size, therapeutic dose, and half-life and to have a chronic treatment for the disease.

Lamivudine is a nucleoside reverse transcriptase inhibitor used in HIV and HBV have been selected for the study to be formulated into a transdermal delivery system. Following oral administration, the elimination half-life is 6.1 hrs; the absolute bio-availability is approximately 80% due to first-pass metabolism and controlled release of the drug is not maintained. So, an alternative route like TDDS was chosen to deliver the drug to the systemic circulation in a controlled manner.

8.0 PLAN OF THE WORK

It was planned to carry out the study in the sequences below.

1. The Compatibility study of drug & polymer by using FT-IR absorption spectra.
2. Preparation of standard curve by using phosphate buffer PH 7.4.
3. Fabrication of Matrix transdermal patches of Lamivudine by using different ratios of hydroxy propyl methyl cellulose (HPMC) and Ethyl cellulose (EC) as polymers.
4. Evaluation of transdermal patches

    - Physico-chemical evaluations
        1. Thickness of the patch
        2. Folding endurance
        3. Percentage of moisture absorbed
        4. Percentage of moisture lost
        5. Water vapor transmission rate
        6. Drug content uniformity
    - Stability studies.
    - *In-vitro* skin permeation studies..
    - Skin irritation test..
    - Statistical analysis by ANOVA
    - *In-vivo* studies.
    - *In-vitro/In-vivo correlation*.

9.0 MATERIALS AND INSTRUMENTS

Drug and its supplier

Table-6

Drug	Supplier /Manufacture
Lamivudine	Aurobindo Pharma Pvt Ltd., Hyderabad , Andhra Pradesh, India

Materials and their supplier

Table-7

Material	Supplier /Manufacture
Hydroxy propyl methyl cellulose- E 15 LV	Loba Chemie Pvt. Ltd., Mumbai.
Ethyl cellulose	Loba Chemie Pvt. Ltd., Mumbai.
Glycerol	Spectrum Reagents and Chemicals Pvt. Ltd., Cochin.
Span 80	Loba Chemie Pvt. Ltd., Mumbai.
Choloroform	Loba Chemie Pvt. Ltd., Mumbai.
Distilled water	Distillation Unit., Banglore.

Instruments and their supplier

Table-8

Instrument	Supplier /Manufacture
Digital Weighing balance	Shimadzu Corporation, Japan.
Dial caliper	Mitutoyo corporation, Japan
pH meter	Hanna instruments, Italy
Orbitek shaker	Scigenics Biotech Pvt. Ltd., Chennai
Franz diffusion cell (60 ml)	Universal Scientific Co., Coimbatore
UV/VIS spectrophotometer	Perkin Elmer, Lambda 25
FTIR spectrophotometer	Perkin Elmer, Spectrum RXI
Stability Chamber	Inlab Equipments, Chennai
HPLC spectrophotometer	Perkin Elmer, Spectrum RXI
Remi CPR-24 centrifuge	Remi instruments, Mumbai
Magnetic stirrer	Bio-Craft Scientific Systems Pvt. Ltd., Agra

10.0 METHODS

10.1 COMPATIBILITY STUDY OF DRUG AND THE POLYMER[80]

FTIR *absorption spectra:* 2 mg of the substance being examined was triturated with 300mg to 400mg of finely powdered and dried potassium bromide. This quantity was usually sufficient to give a disc of 13 mm diameter and a spectrum of suitable intensity. The mixture was grinded carefully, spread it uniformly in a suitable die, and submit in a *vacuo to a pressure of about 800 Mpa* ($8t.cm^{-2}$). The same product was repeated for the polymer and the physical mixture of drug and the polymers.

10.2 REAGENTS PREPRATION

Preparation of phosphate buffer pH 7.4 [62]

Place 50.0ml of 0.2M potassium dihydrogen phosphate in a 200ml volumetric flask, add the specified volume of 39.1 ml of 0.2M sodium hydroxide and then add water to make up the volume.

0.2M potassium dihydrogen phosphate

Dissolve 27.218g potassium dihydrogen phosphates in distilled water and dilute to 1000ml with distilled water.

0.2M sodium hydroxide solution

Dissolve 8g of sodium hydroxide in distilled water and dilute to 1000ml with distilled water.

10.3 PREPARATION OF STANDARD CURVE[5]

Stock solution

100mg of Lamivudine was dissolved in 100ml of pH 7.4 phosphate buffer, so as to get a solution 1000µg/ml concentration.

Standard solution

10 ml of stock solution was made to 100ml with pH 7.4 thus giving a concentration of 100 µg/ml. Aliquot of standard drug solution ranging from 1ml to 8ml were transferred in

to 10ml volumetric flask and were diluted up to the mark with pH 7.4 phosphate buffer. Thus the final concentration ranges from 10-60 µg/ml. Absorbance of each solution was measured at 270 nm against pH 7.4 phosphate buffer as a blank. A plot of concentrations of drug versus absorbance was plotted.

Normal saline

Dissolve 0.9% w/v sodium chloride in 100 ml of purified water.

10.4 DOSE DESIGNING[81]

On the basis of pharmacokinetic parameters of Lamivudine, the drug delivery rate through skin required to achieve effective plasma concentration was calculated using the equation,

$$K_p = Q/t = (C_{plasma})_{ss}. K_{el} V_d$$

Where, Q/t (or) K_p is the drug input rate

$(C_{plasma})_{ss}$ is the drug level at steady state

K_{el} is elimination rate constant

V_d is the volume of distribution of drug

In calculation of dose designing for the formulation of transdermal drug delivery system, the skin/drug partitioning and polymer/drug partitioning are play major role.

Table-9

$(C_{plasma})_{ss}$ (µg/L)	K_{el} $(h)^{-1}$	V_d (L/kg)	K_p (mg/hr)	Total drug/24hrs (mg)
1.4	0.1136	1.3	0.2067	4.96 mg. ≅ 5.0 mg

10.5 FABRICATION OF TRANSDERMAL PATCHES [63,64,65,66]

Matrix patches were casted on glass mould by solvent casting method. Four types of polymer patches were prepared. First two formulations were prepared by using HPMC alone having drug & polymer ratio 1**:**2.5, 1**:**5 using water as a solvent and the next formulations were formulated using EC having drug & polymer ratio 1**:**2.5, 1**:**5 using chloroform as a solvent with Span 80 (1%), as permeation enhancer, Glycerol (3%) was used as a plasticizer. The films were cast onto a suitably designed and fabricated glass mould and then dried in hot air oven at 40^0C for six hours . The films were removed by using sharp blade by inserting along the edges of the film. The dried films were wrapped in aluminium foils and stored in a closed container in cool place away from light.

Table-10**: Fabrication of Lamivudine Matrix Transdermal patches of 3.14 Sq cm**

S. No	Ingredients	F_1 W (1:2.5)	F_2 W (1:5)	F_3 W (1:2.5)	F_4 W (1:5)	FPE_1 (1:2.5)	FPE_2 (1:5)	FPE_3 (1:2.5)	FPE_4 (1:5)
1	Drug (Lamivudine in mg)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
2	Hydroxy propyl methyl cellulose (mg)	12.5	25	--	--	12.5	25	--	--
3	Ethyl cellulose (mg)	--	--	12.5	25	--	--	12.5	25
4	Span-80	--	--	--	--	1%	1%	1%	1%
5	Glycerol	3%	3%	3%	3%	3%	3%	3%	3%

10.6 PHYSICOCHEMICAL EVALUATIONS OF THE TRANSDERMAL PATCHES [64, 67,68,69,70]

Thickness of the patch

Thickness of the patch was measured by using 'Dial gauge' in mm.

Folding endurance

The folding endurance was measured manually for the prepared film. A strip of film was cut evenly and folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance.

Percentage of moisture absorbed [71]

To check the physical stability of the film in high humidity condition, accurately weighed film were placed in a desiccators containing saturated solution of Aluminium chloride (79.5% relative humidity) for 3 days. The films were reweighed and percentage moisture absorption was calculated using the formula.

$$\text{Percentage moisture absorption} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Percentage of moisture lost[71]

To check the extent of moisture loss from freshly prepared film, accurately weighed films were placed in a desiccator containing fused anhydrous Calcium chloride for 72 hrs. After 72 hrs, the films were reweighed and percentage moisture loss was calculated using the formula.

$$\text{Percentage moisture lost} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Water vapor transmission[68, 72, 73]:

It is defined as the quantity of moisture transmitted through unit area of film in unit time. For the study of WVT rate, vials of equal diameter were used as transmission cells (approximately 5 ml vials). These cells washed thoroughly and dried in an oven. Approximately 1g of calcium chloride was taken in the cell and the polymeric films measuring 3.14 cm^2 areas were fixed over the brim with the help of an adhesive. The cells were weighed accurately and initial weight is recorded, and then kept in a closed desiccator containing saturated solution of potassium chloride (approximately 200 ml). The humidity inside the desiccator was measured by a hygrometer, and it was found to be in between 80% and 90% RH. The cells were taken out and weighed after 18, 36, 54, and 72 hours.

From increase in weights, the amount of water vapor and the rate at which water vapor transmitted was calculated by using the following formula.

$$\text{WVT rate} = \frac{WL}{S}$$

Where, W = water vapor transmitted (in g)

L = thickness of the film (in cm)

S = exposed surface area (in cm^2)

WVT through films determines the permeability characters of the films.

Drug content uniformity[64]

The prepared patch was cut into small pieces and put into 100 ml dissolution or diffusion medium used respectively and stirred continuously using a mechanical stirrer and sample was with drawn at the end of three hours and the drug content was determined, spectrophotometrically at 270 nm.

Stability study[64, 67]

Stability of a product may be defined as the capability of particular formulation in a specific container to remain with the physical, chemical, microbiological, therapeutic and toxicological specifications. The stability studies were conducted according to ICH guidelines. The prepared patches were stored for three months at different temperatures 40°C ± 2° C temperature and at relative humidity of 75% ± 5% RH. The stability study was conducted with regard to tensile strength, moisture content and drug content. The patches, which retained their physical properties, were further subjected to *in-vitro* permeation studies.

10.7 *IN VITRO* SKIN PERMEATION STUDIES OF TRANSDERMAL PATCH [64, 67, 69, 74]

Preparation of excised Albino Rat skin

Albino rats weighing 150-200 g were selected for permeation studies and the study was conducted with the approval of institutional ethical committee. The Rat was sacrificed using anesthetic ether, hair of test animal was carefully trimmed short (<2 mm) with a pair of scissors. Approximately 4.5 to 5.0 Sq cm of full thickness of skin was excised from the shaved abdomen site. After removal of the subcutaneous fat; the skin sample was washed in distilled water. The separated skin was cut into required size and placed in polythene bag and stored under freezing condition into the refrigerator until taken for *in vitro* permeation studies.

In vitro skin permeation studies

Ethical clearance for the handling of experimental animals was obtained from the institutional animal ethical committee (IAEC) formed for this purpose. The protocol of the animal study was approved by the Institutional Animal Ethical committee, Protocol Number: **SVCP/IAEC/M.Pharm/03/2009 dt 07.11.09**. The experiment was conducted according to the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiment on animals).

These studies were carried out using a mammalian rat skin of required thickness. The permeation cell used for this study was a specially fabricated “Modified Franz diffusion cell”.

Modified Franz diffusion cell [75,76,77]

Modified Franz diffusion cell is a skin permeation system developed by Franz and commercialized by crown glass has been frequently used for the studying the kinetics of Percutaneous absorption. The modified Franz diffusion cell has receptor compartment with an effective volume approximately 60 ml and an effective surface area of permeation of 3.14 sq cms. A rod shaped magnetic stirrer driven by a 3w synchronized motor stirs the solution in the receptor compartment. The stirring magnet rotates at constant rpm in a low viscosity receptor compartment.

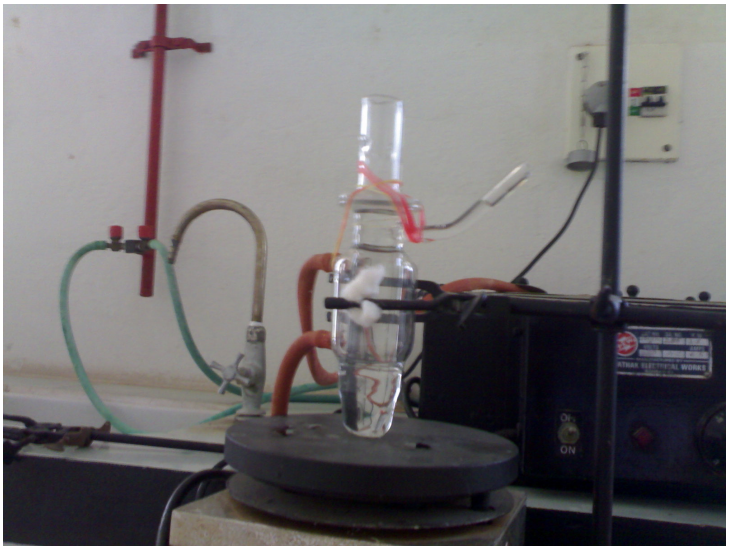


Fig 13**: Modified Franz diffusion cell**

Operation of diffusion cell

The skin was mounted and clamped between the receptor and donor compartments. The surface area exposed to the drug patch was 3.14 sq cm. The cell was maintained at 37°±0.5°C using thermostatically controlled water bath. Water from the water bath was circulated through the cell, which was maintained at constant flow. The diffusion cell of 60

ml capacity was filled with phosphate buffer pH 7.4 and fixed to a thermostatically controlled magnetic stirrer, which was also maintained at the temperature 37° ±0.5°C.

At each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 270 nm.

10.8 KINETIC CHARACTERISTICS OF THE DRUG RELEASE [78, 79]

To know the mechanism of the drug release from the patches, the results obtained from the *In-vitro* dissolution process were fitted into different kinetic equations as follows:

1. Zero - order drug release: Cumulative % drug release Vs Time.
2. First Order drug release: Log cumulative % drug retained Vs Time.
3. Higuchi's classical diffusion equation: Cumulative % drug release Vs Square root of time.
4. Peppa's Korsemeyer Exponential equation: Cumulative % drug release Vs Log time.

1. Zero order kinetics

Zero order release would be predicted by the following equation:-

$$A_t = A_0 - K_0 t$$

Where, A_t = Drug release at time't'.

A_0 = Initial drug concentration.

K_0 = Zero - order rate constant (hr^{-1}).

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero – order equal to K_0.

2. First Order Kinetics

First – order release would be predicted by the following equation:-

$$\text{Log } C = \log C_0 - Kt / 2.303$$

Where,

C = Amount of drug remained at time‘t’.

C_0 = Initial amount of drug.

K = First – order rate constant (hr^{-1}).

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follow first order kinetics. The constant ‘K’ can be obtained by multiplying 2.303 with the slope values.

3. Higuchi’s model

Drug release from the matrix devices by diffusion has been described by following Higuchi’s classical diffusion equation: -

$$Q = [D\varepsilon / \tau (2 A - \varepsilon Cs) Cst]^{½}$$

Where,

Q = Amount of drug released at time‘t’.

D = Diffusion coefficient of the drug in the matrix.

A = Total amount of drug in unit volume of matrix.

Cs = the solubility of the drug in the matrix.

ε = Porosity of the matrix.

τ = Tortuosity.

t = Time (hrs) at which ‘q’ amount of drug is released.

Above equation may be simplified if one assumes that ‘D’, ‘Cs’ and ‘A’ are constant. Then equation becomes:- $Q = Kt^{1/2}$

When the data is plotted according to equation i.e. cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to ‘K’.

4. Korsemeyer equation / Peppa’s model

To study the mechanism of drug release from the transdermal patches, the release data were also fitted to the well-known exponential equation (Korsemeyer equation/ Peppa’s law equation), which is often used to describe the drug release behavior from polymeric systems.

$$M_t / M_\alpha = Kt^n$$

Where,

M_t / M_α	=	The fraction of drug released at time‘t’.
K	=	Constant incorporating the structural and geometrical characteristics of the drug / polymer system.
n	=	Diffusion exponent related to the mechanism of the release.

Above equation can be simplified by applying log on both sides,

$$\text{Log } M_t / M_\alpha = \text{Log } K + n \text{ Log } t$$

When the data is plotted as log of drug released versus log time, yields a straight line with a slope equal to ‘n’ and the ‘K’ can be obtained from y – intercept. For Fickian release ‘n’ = 0.5 while for anomalous (non - Fickian) transport ‘n’ ranges between 0.5 and 1.0.

‘n’ values can be used to characterize diffusion release mechanism as :

n < 0.5	Fickian diffusion
0.5 > n < 1	Non-fickian diffusion
n > 1	Class – II transport

THE STUDY OF *IN VITRO* SKIN PERMEATION KINETICS[81, 82]:

Permeability coefficient (P): Permeability coefficient is the velocity of drug passage through the membrane in mg/cm^2/day. It was calculated from the slope of the graph of percentage of drug transported versus time as,

$$P = \text{slope} \times Vd/S$$

Where, Vd = volume of donor solution;

S = surface area of tissue.

Flux (J): Flux is defined as the amount of material flowing through a unit cross-sectional barrier in unit time. It is calculated by,

$$J = P \times CD$$

Where, CD = concentration donor solution; P = permeability

Enhancement ratio (E_r): Enhancement ratio was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules. It is calculated by,

$$\text{Enhancement ratio} = \frac{\text{Permeability coefficient of drug with enhancer}}{\text{Permeability coefficient of drug alone}}$$

.

10.9 SKIN IRRITATION TEST FOR PATCHES[67, 68, 69, 83]

Ethical clearance for the handling of experimental animals was obtained from the institutional animal ethical committee (IAEC) formed for this purpose. The protocol of the animal study was approved by the Institutional Animal Ethical committee, Protocol Number: **SVCP/IAEC/M.Pharm/03/2009 dt 07.11.09**. The experiment was conducted

according to the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiment on animals).

The skin irritation test was done on a healthy rabbit weighing between 2 to 3 kg. The rabbits were to be divided into 5 groups with three each. On the previous day of the experiment, the hair on the back side of the rabbit is to be removed. Drug loaded polymeric film of 3.14 sq cm was placed on the left dorsal surface of the rabbit. The skin was examined for erythema/edema.

Group – I **:** Served as normal, without any treatment

Group – II **:** Control- applied with marketed official adhesive tape in USP

Group – III **:** Blank – applied with drugless polymeric patch

Group – IV **:** Test – applied with drug loaded polymeric patch

Group – V **:** Applied with 0.8% v/v aqueous solution of formalin solution as standard irritant.

The animals were applied with new patch/formalin solution each day up to 7days and finally the application sites were to be graded according to a visual scoring scale.

The erythema scale is as follows:

0. none
1. slight
2. well defined
3. moderate
4. scar formation

The edema scale is as follows:

0. none
1. slight
2. well defined
3. moderate
4. severe

Fig 14: **Skin irritation test on rabbit**

10.10 STATISTICAL ANALYSIS BY ANOVA

The in-vitro permeation data were statistically compared and analyzed using Graph Pad In stat – 3 software version. The Tukey – Kramer multiple comparisons test was utilized to find the 'q' and 'p' values and the significance of the formulations were studied.

If the 'p' is <0.05, the formulations are said to be significant

10.11 *IN-VIVO* ANIMAL STUDIES[84]

Six Wister rats of 180-220gm weight are to be utilized for the study. The rats are to be allowed to acclimatize for 1 week before the day of administration. The rats are fasted, but allowed access to water on the day before the study

Standard concentration solution of lamivudine[5]

A mixture of acetonitrile and water (65:35%V/V) was used as the mobile phase and C_{18} column as the stationary phase. A stock solution of the pure drug was prepared by dissolving 50 mg of lamivudine in 50ml of the mobile phase. This stock solution was further diluted with the mobile phase to obtain the concentration of 10µg/ml. six samples of this concentration are prepared. And observed in HPLC.

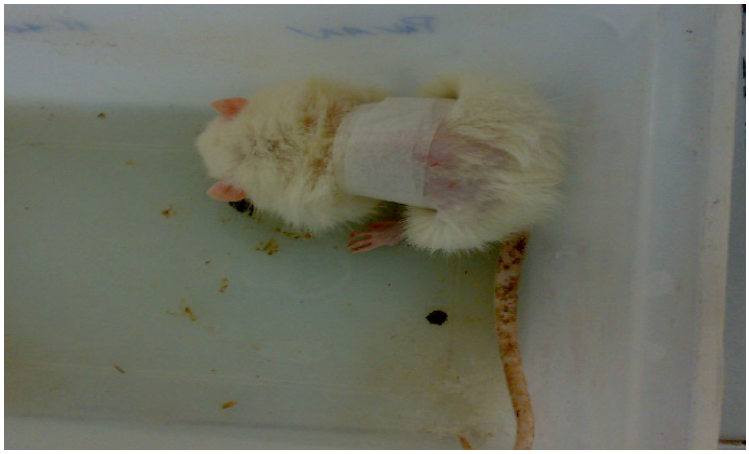


Fig: 15 **Transdermal patch on rat abdomen**

Six groups of rats are taken for animal studies. Each group containing one rat. Rats are to be anesthetized before the experiment. The hair on the abdominal site is clipped before the experiment. The skin was gently wiped between warm water and alcohol swab and patted dry. 3.14sq.cm size patch with 5mg amount of drug is to be fixed over the skin. Blood samples of approximately 0.15 ml were collected in dried heparinzed tubes at 0.5, 2, 4, 8, 16 and 24hr after transdermal administration from the jugular vein and, as soon as possible, they are frozen at -20ºC and store until analysis.

The HPLC analytical reports of blood samples collected at different hours were compared with that of the standard concentration of lamivudine.

10.12 IN-VITRO / IN-VIVO CORRELATION[85]

Invitro-invivo correlation is the demonstration of the direct relationship of *in vitro* dissolution rate / diffusion rate of drugs and their *in vivo* bioavailability. Generally, the *in vitro* property is the rate or extent of drug dissolution or release while the *in vivo* response is the plasma drug concentration or amount of drug absorbed[86]. Correlation is used to ensure batch-to-batch consistency in the physiologic performance of a drug product by use of such *in vitro* values and to serve as a tool in the development of a new dosage form with desired *in vivo* performance.

There are two basic approaches by which a correlation between dissolution/diffusion testing and bioavailability can be developed.

1. By establishing a relationship, between the *in vitro* dissolution/diffusion and the *in vivo* bioavailability parameters. If this relationship becomes linear with a slope of 1, then curves are super imposable, and there is a 1:1 relationship which is defined as point-to-point or level A correlation.

2. By using the data from previous bioavailability studies to modify the dissolution/diffusion methodology in order to arrive at meaningful *in vitro -invivo correlation*.

11.0 RESULTS

Transdermal Matrix patch was prepared with the aim to reduce first pass metabolism and there by increasing the bioavailability of the drug. It also reduces the frequency of dosing which in turn improve patient compliance and reduce fluctuation in plasma drug levels.

The calibration curve was prepared with phosphate buffer pH 7.4. The physical mixture of Drug, polymer and the mixture of drug and polymers were subjected to compatibility study using *FTIR absorption spectra.* The fabricated transdermal patches were subjected to various evaluation parameters like, Thickness of the patch, Folding endurance, Percentage of moisture absorption, Percentage of moisture loss, Water vapor transmission, Drug content uniformity, Stability studies, *In-vitro* skin permeation studies, Skin irritation test, Statistical analysis by ANOVA, *In-vivo* studies and *In-vitro* & *In-vivo* correlation.

Compatibility study

The drug was identified and confirmed by FTIR spectrum. Fig-16 showed the IR spectrum of Lamivudine. Fig-17 shows the IR spectrum of hydroxy propyl methyl cellulose. Fig-18 shows the IR spectrum of ethyl cellulose. Fig-19 shows the physical mixture of Lamivudine and hydroxy propyl methyl cellulose. Fig-20 shows the physical mixture of Lamivudine and ethyl cellulose.

From the Fig-19 and Fig-20, it were concluded that the drug along with the polymers showed no change in any characteristic peak of the drug, which confirms that there is no interaction between the drug and the polymer used in the fabrication of transdermal patch. The presence of peaks at the expected range confirms that the materials taken for the study are genuine.

Characteristic peaks of drug in FT-IR spectra

Table-11

S. No	Wave in cm^{-1}	Functional group
1	3076.75	C-H Aromatic stretching
2	3213.19	NH_2 group
3	1650.95	Carbonyl group
4	3326.96	-OH group
5	1317.29	C-O Bending group
6	626.82	C-S Bending group

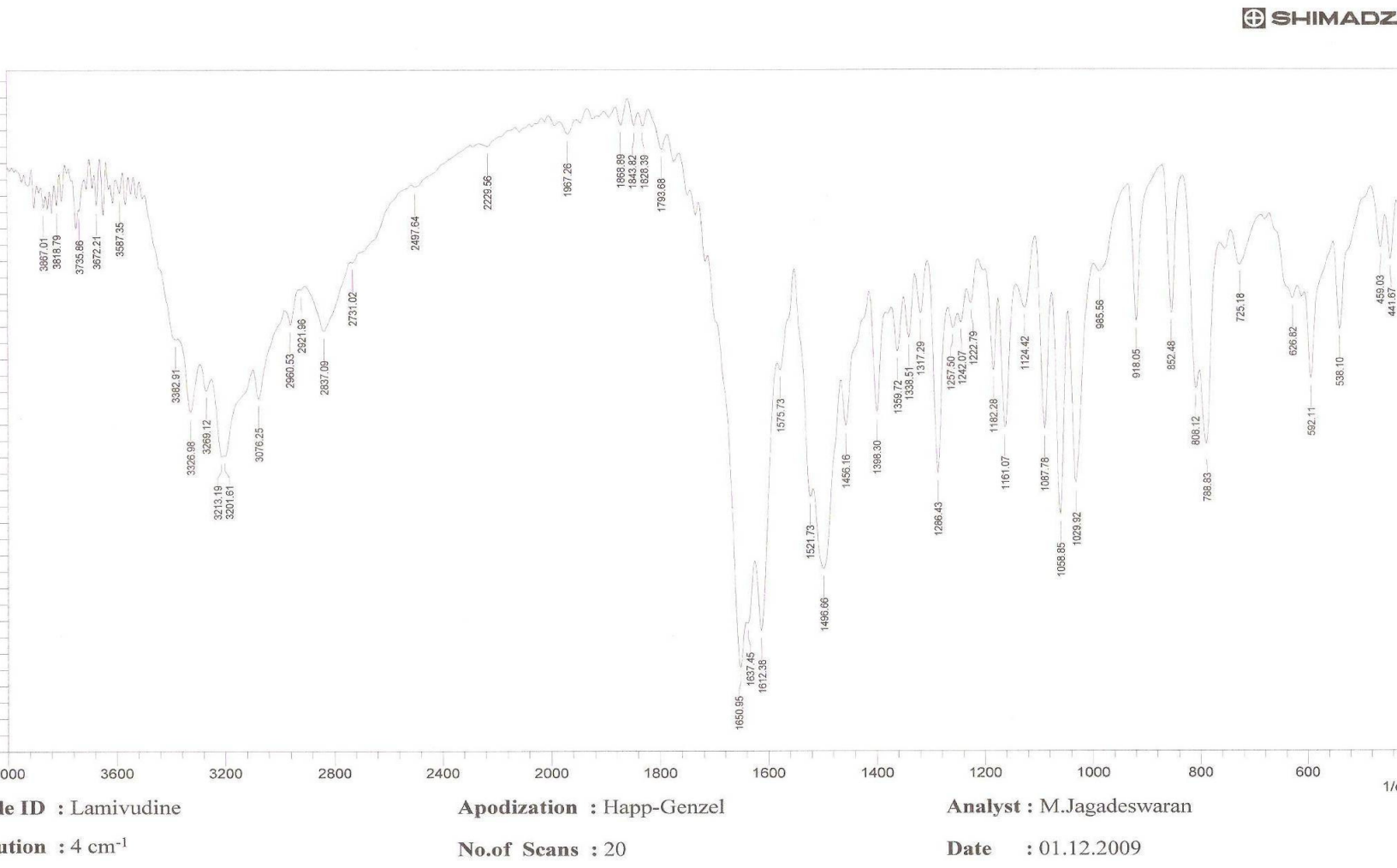


Fig. 16

FT-IR spectra of Pure Lamivudine.

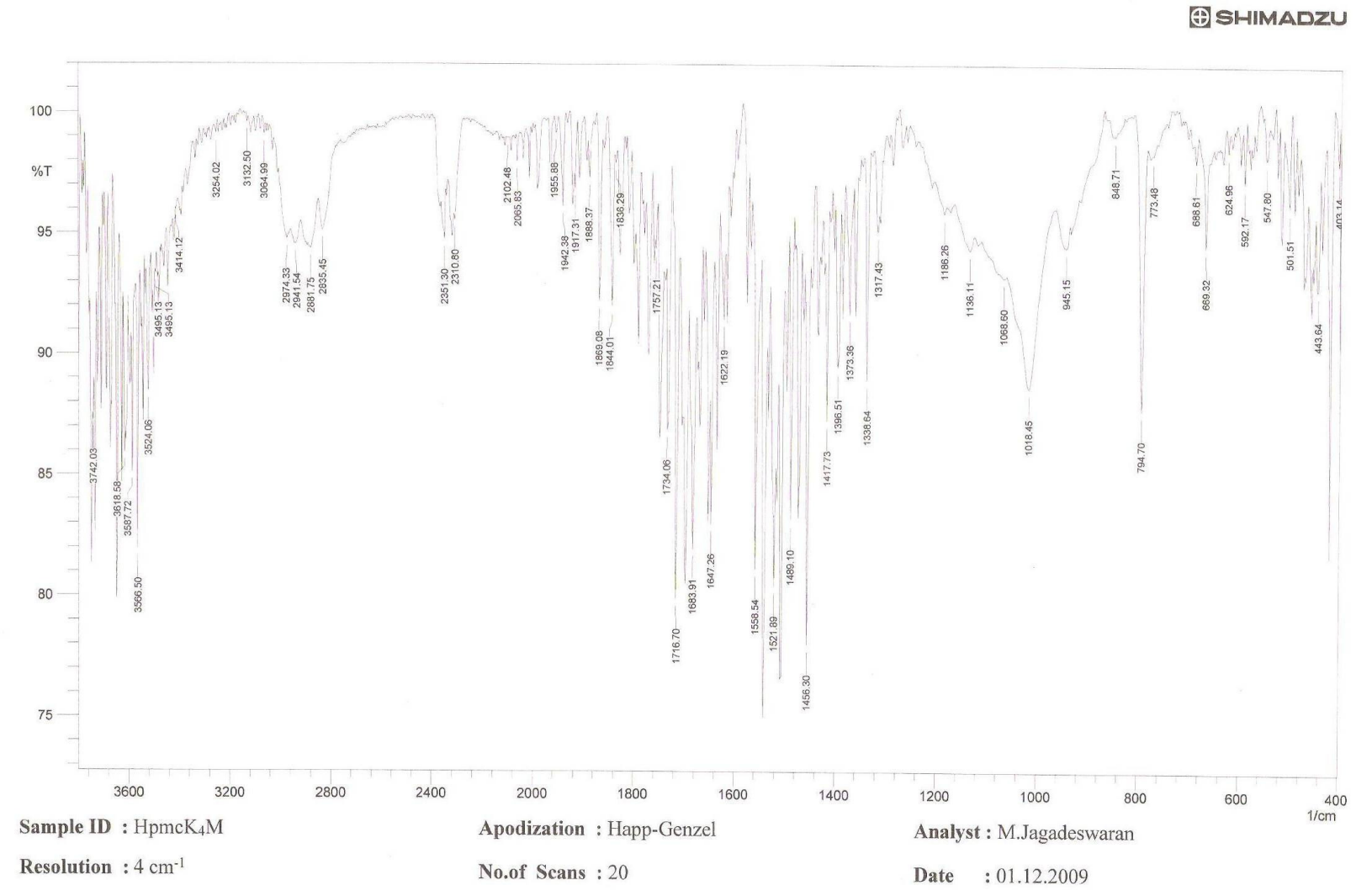


Fig. 17
FT-IR spectra of HPMC K4M

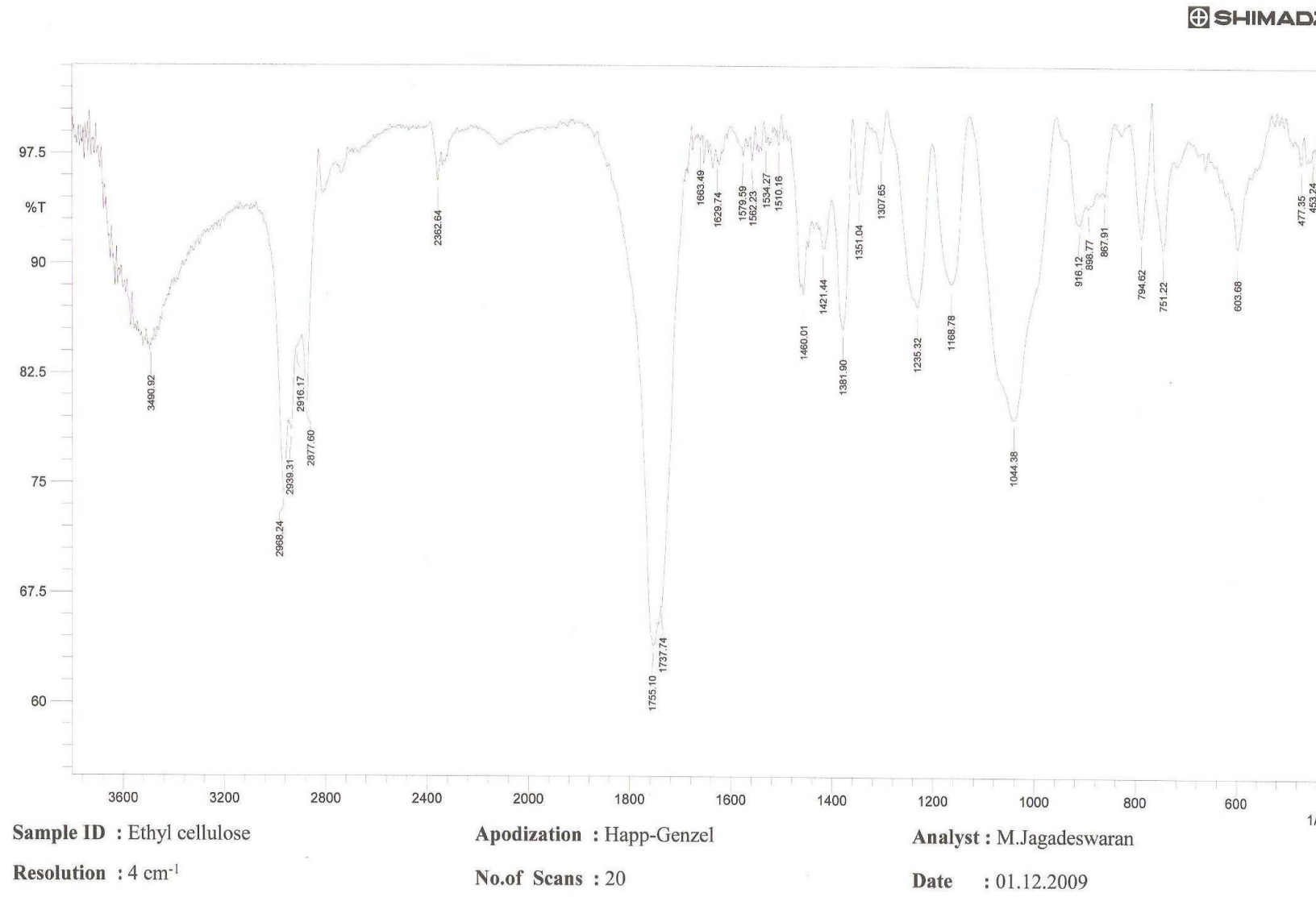


Fig-18: **FT-IR spectra of Ethyl cellulose**

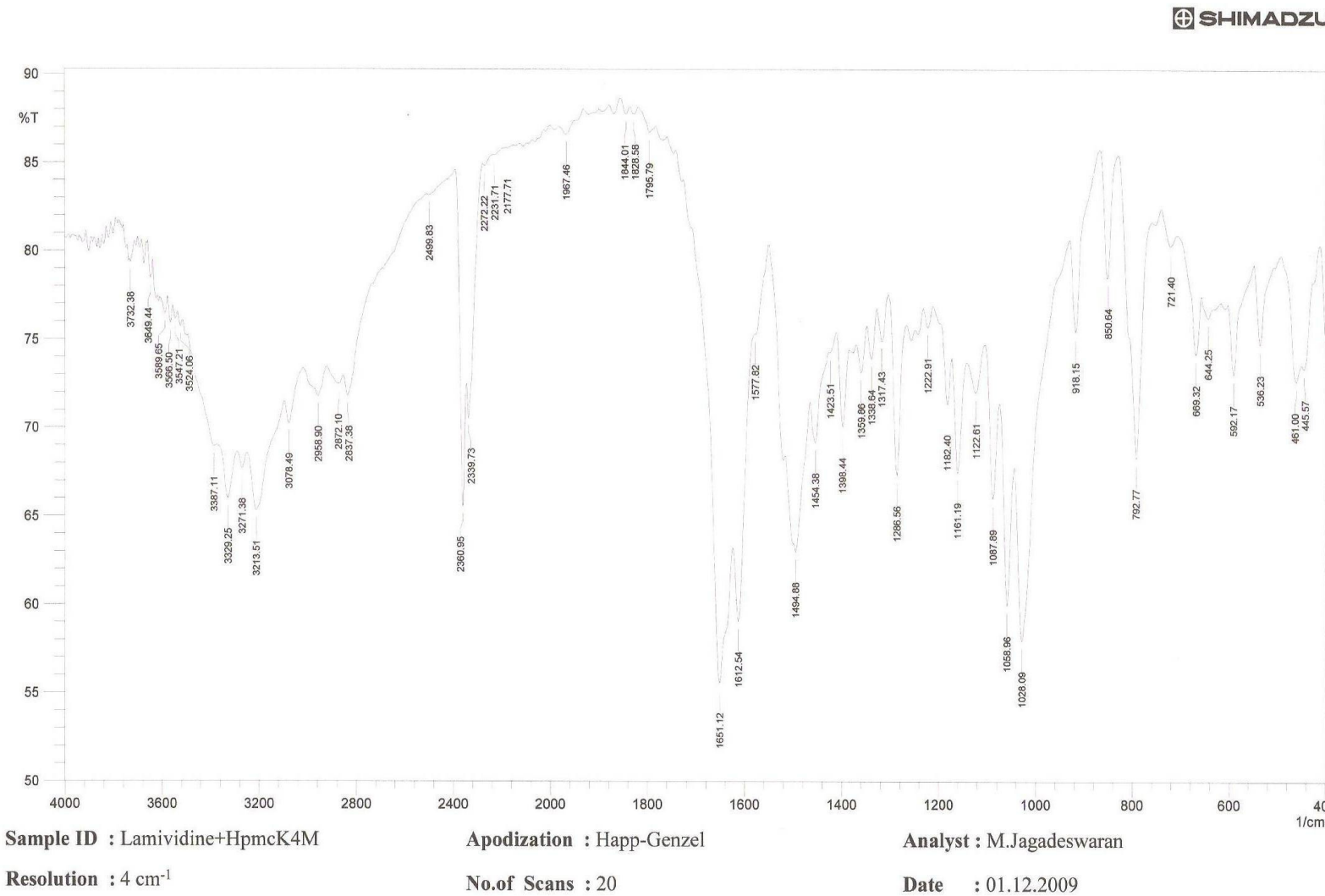


Fig-19: **FT-IR spectra of physical mixture of Lamivudine and HPMC K4M**

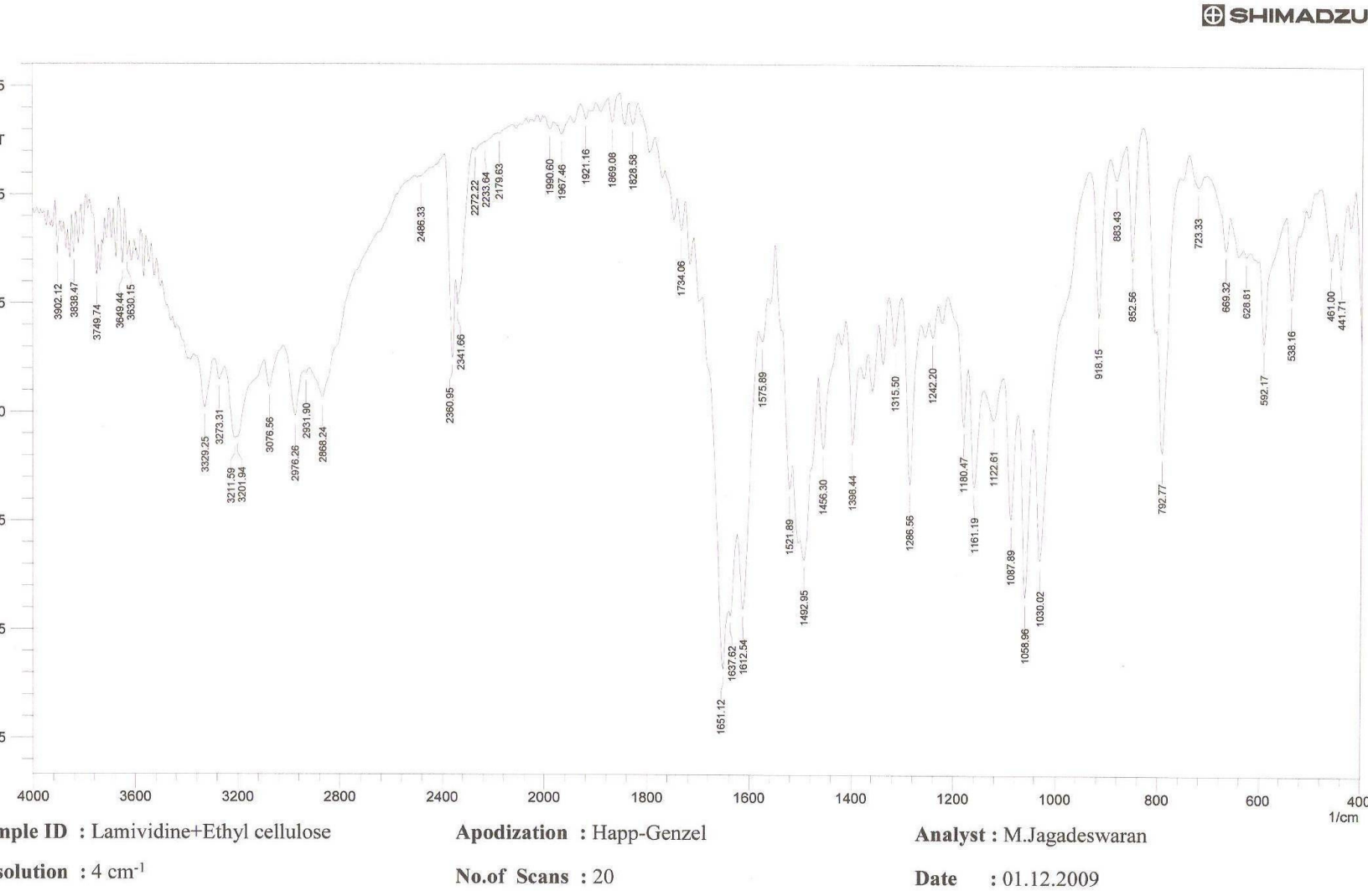


Fig-20: **FT-IR spectra of physical mixture of Lamivudine and Ethyl cellulose**

Standard curve of Lamivudine

Table no.13 and Fig-19 shows the standard curve for Lamivudine in phosphate buffer pH 7.4. The method obeyed Beer's law limit in the concentration range of 10-60 mcg/ml at 270 nm.

Standard Curve

Table-12

Concentration (mcg/ml)	Absorbance at 270 nm
0	0
10	0.103
20	0.200
30	0.291
40	0.380
50	0.474
60	0.560

Slope **:** 0.042261

Regression**:** 0.998248

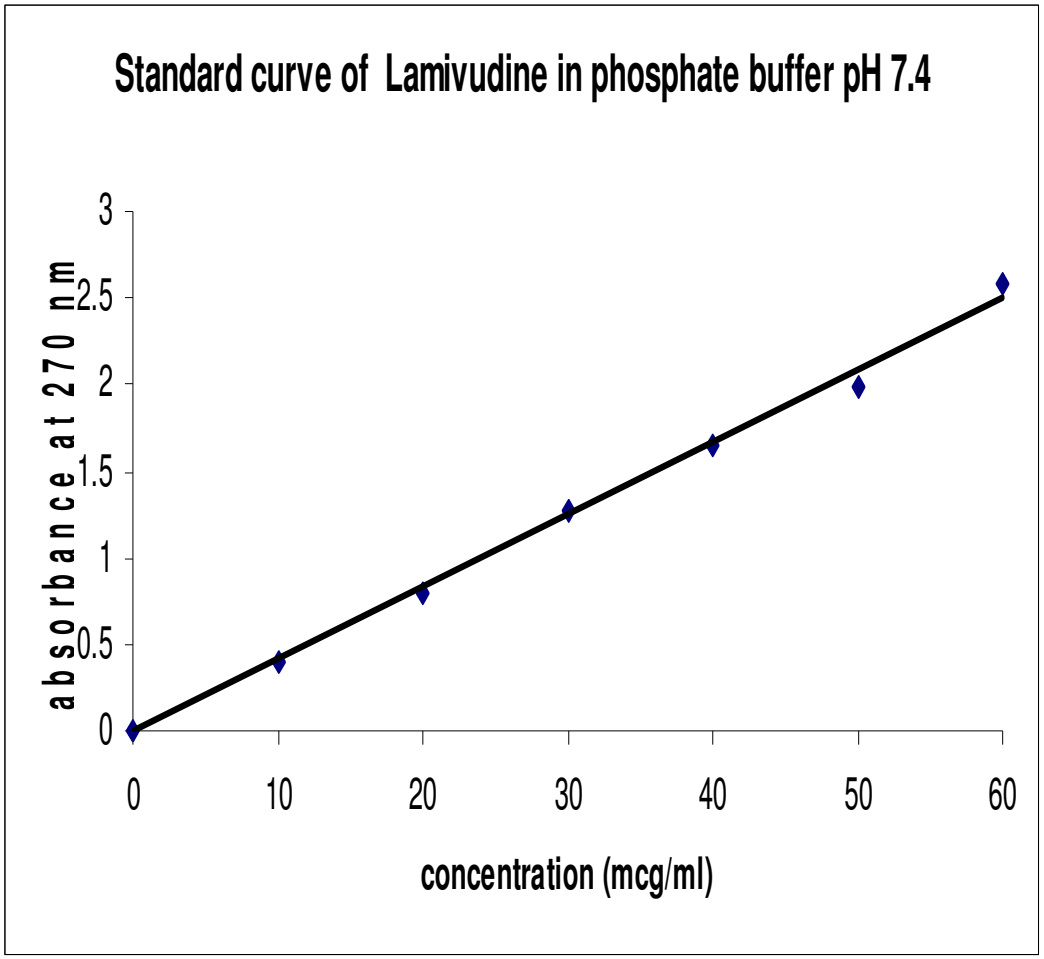


Fig: 21 **Standard curve of Lamivudine.**

Physicochemical Evaluation of the prepared Transdermal patches

Table 13 and 14 shows the physicochemical evaluation like the Thickness, Folding endurance, Percentage moisture absorbed, Percentage moisture lost, Drug content uniformity.

Table 13**: Physicochemical Evaluations**

Formulation Code	***Thickness (mm)**	***Folding endurance**	***% Moisture absorbed**	***% Moisture lost**	***Water vapor transmission rate**
F_1	0.250±0.85	140±1.4	2.88±0.80	1.20±0.50	0.112±0.002
F_2	0.282±0.65	124±1.8	2.32±0.70	1.10±0.75	0.125±0.001
F_3	0.323±0.90	98±1.0	1.95±1.10	1.05±0.90	0.134±0.004
F_4	0.345±1.0	160±1.2	2.78±1.50	1.45±1.00	0.153±0.005

*Average of three values
± Standard deviation

Table- 14**: Drug content uniformity**

Formulation Code	**Percentage of drug in 3.14 Sq. cms**						**Standard Deviations**
	1	**2**	**3**	**4**	**5**	***Mean**	
F_1	98.2	98.4	98.6	98.4	98.2	98.3	±0.16
F_2	97.5	98.2	97.2	97.8	98.4	97.8	±0.49
F_3	95.6	96.2	95.1	95.9	96.6	95.8	±0.57
F_4	92.7	92.1	94.7	93.9	93.2	93.3	±1.01

*Average of five values
± Standard deviations

Stability study

The stability studies conducted reveled that polymeric patches of Lamivudine prepared with the polymers, Hydroxy propyl methyl cellulose and Ethyl cellulose in the ratios of 1:2.5 and 1:5 with 3% plasticizer, Glycerol were found to be most stable with respect to drug content and all the physical parameters evaluated (Table-13 & 14). Hence, the patches were suitable for further studies.

IN VITRO SKIN PERMEATION STUDIES OF TRANSDERMAL PATCH

Table- 15 ***In vitro*** **Skin Permeation of control formulation-F_1W (Without permeation enhancer)**

Time (hrs)	Cumulative % of Drug permeation			*Mean	Standard deviations (±SD)
	Trial I	Trial II	Trial III		
0	0	0	0	0	0
0.25	0.56872	0.511848	0.654028	0.578199	0.071562
0.5	1.43128	1.37346	1.518009	1.440916	0.072755
0.75	2.592417	2.533649	2.680569	2.602212	0.073948
1	4.341232	3.769668	5.198578	4.436493	0.719202
2	6.402844	5.821801	7.274408	6.499684	0.731129
4	8.781991	8.191469	9.667773	8.880411	0.743056
6	12.19431	11.60379	13.08009	12.29273	0.743056
8	18.50711	17.89763	19.42133	18.60869	0.766911
10	23.92417	23.30521	24.85261	24.02733	0.778838
12	27.72038	27.09194	28.66303	27.82512	0.790765
14	31.2891	30.65118	32.24597	31.39542	0.802692
16	36.61611	35.96872	37.5872	36.72401	0.814619
18	42.30806	41.65118	43.009	42.32275	0.679029
20	45.52607	44.85972	46.52085	45.63555	0.835963
22	47.93365	47.25782	48.94265	48.04471	0.84789
24	51.78199	51.09763	52.80379	51.89447	0.858624

*Average of three values
± Standard deviations

Fig 22**: *In vitro* skin permeation of formulation –F_1W**

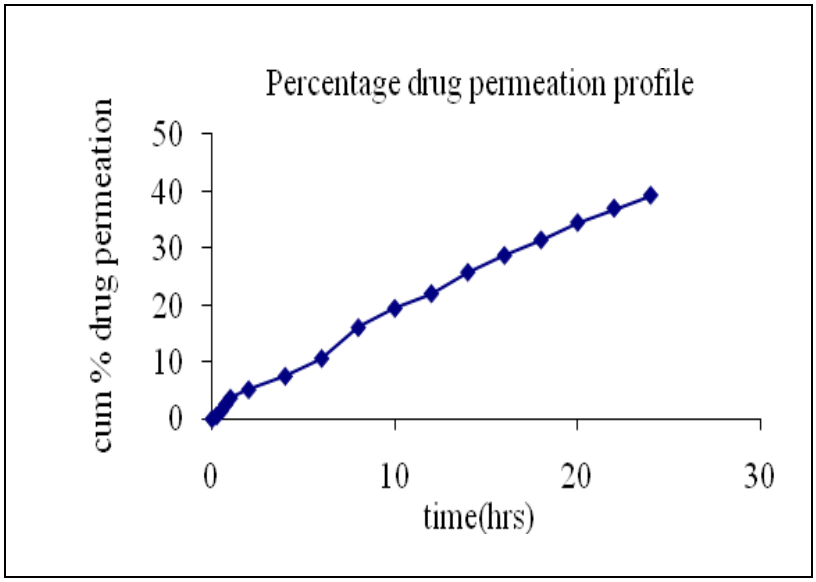


Fig 23**: Zero order permeation profile of formulation F_1 W**

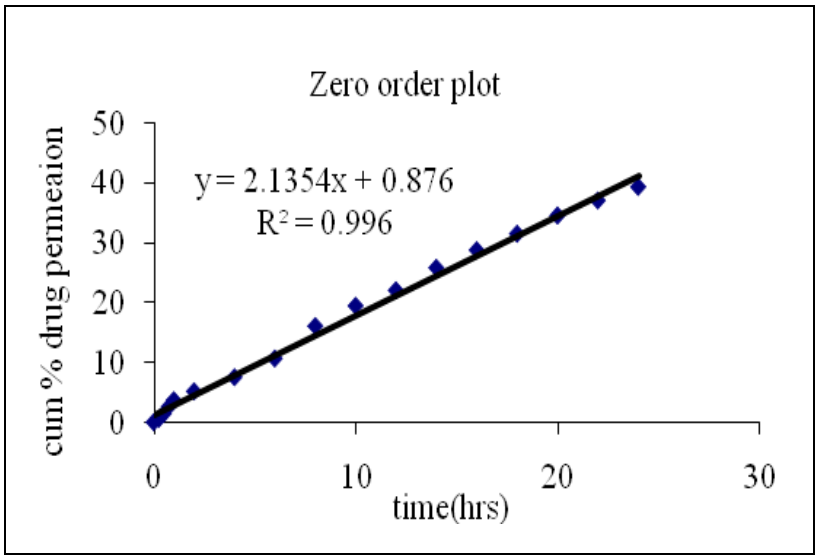


Fig 24: **First order permeation profile of formulation F_1 W**

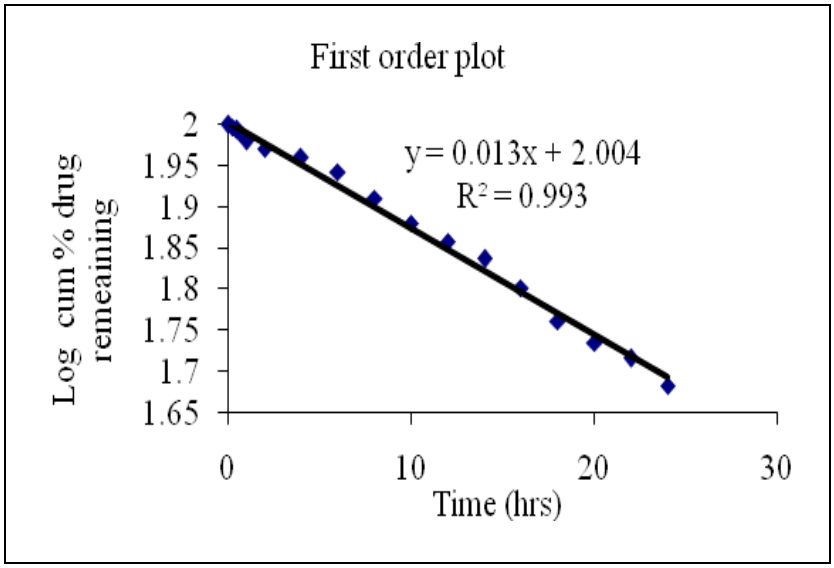


Fig 25: **Higuchi permeation profile of formulation F_1**

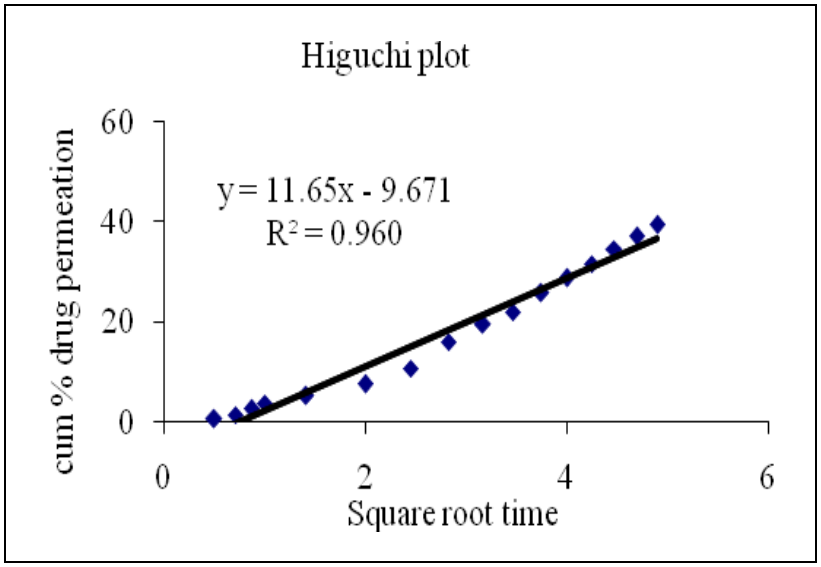


Fig 26: **Korsemeyer peppa's permeation profile of formulation F_1 W**

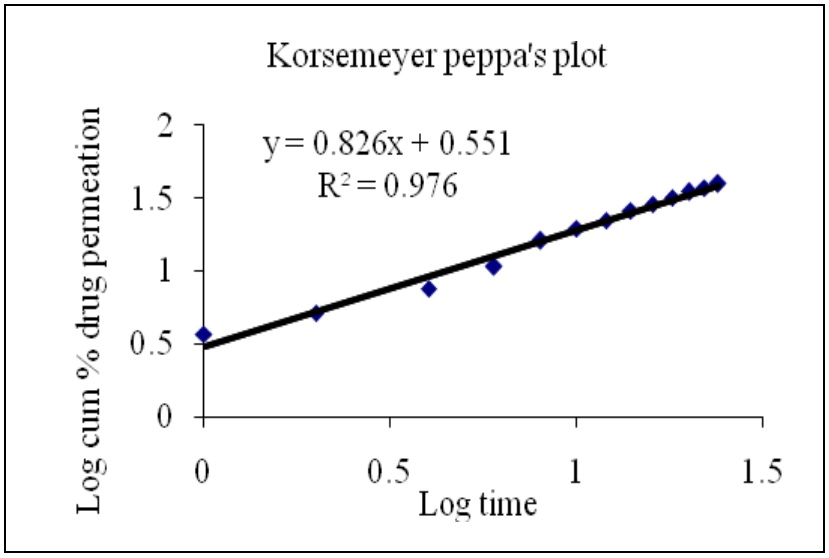


Table- 16 ***In vitro*** **Skin Permeation of control formulation- F_2 W**

(Without permeation enhancer)

Time (hrs)	Cumulative % of Drug permeation			*Mean	Standard deviations (±SD)
	Trial I	Trial II	Trial III		
0	0	0	0	0	0
0.25	3.127962	2.559242	3.412322	3.033175	0.434367
0.5	8.014218	7.436019	8.303318	7.917852	0.441607
0.75	11.55924	10.97156	11.85308	11.4613	0.448846
1	14.87678	14.27962	15.17536	14.77725	0.456086
2	18.81517	18.20853	19.11848	18.71406	0.463325
4	21.96209	21.34597	22.27014	21.8594	0.470565
6	25.37441	24.75829	25.68246	25.27172	0.470565
8	28.69194	28.05687	29.00948	28.5861	0.485044
10	32.5545	31.90995	32.87678	32.44708	0.492283
12	37.04265	36.38863	37.36967	36.93365	0.499522
14	41.59716	40.93365	41.92891	41.48657	0.506762
16	43.94313	43.27014	44.27962	43.83096	0.514001
18	48.87678	48.19431	49.21801	48.76303	0.521241
20	51.32227	50.63033	51.66825	51.20695	0.52848
22	54.08057	53.37915	54.43128	53.96367	0.53572
24	56.87204	56.16114	57.22749	56.75355	0.542959

*Average of three values

± Standard deviations

Fig 27**: *In vitro* skin permeation of formulation – F_2 W**

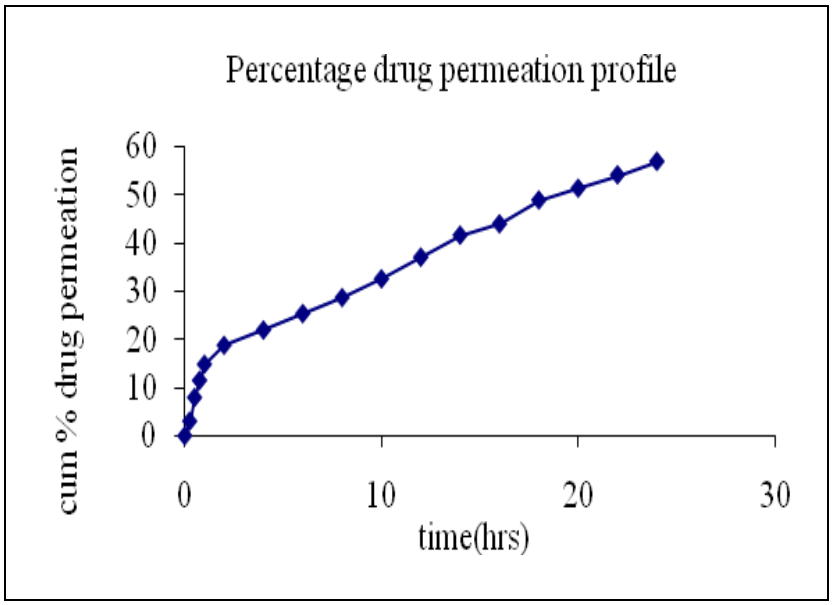


Fig 28: **Zero order permeation profile of formulation F_2 W**

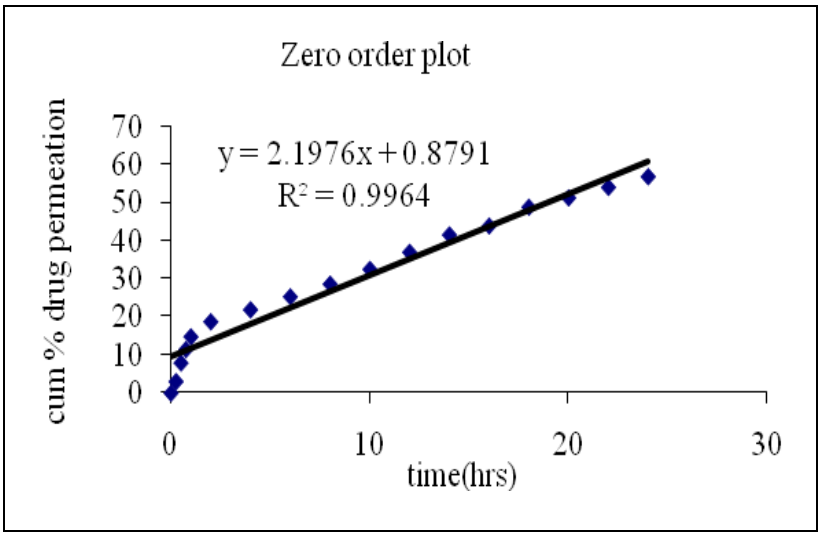


Fig 29: **First order permeation profile of formulation F_2 W**

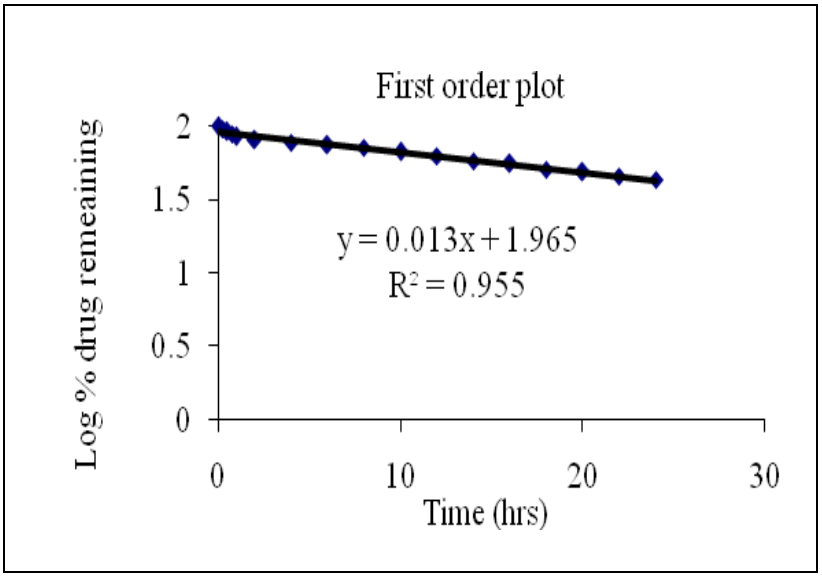


Fig 30: **Higuchi permeation profile of formulation F_2 W**

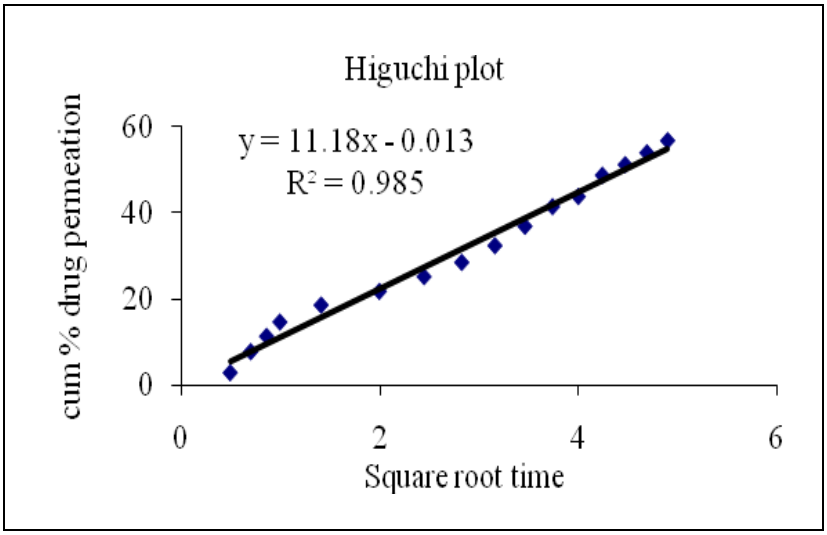


Fig 31: **Korsemeyer peppa's permeation profile of formulation F_2 W**

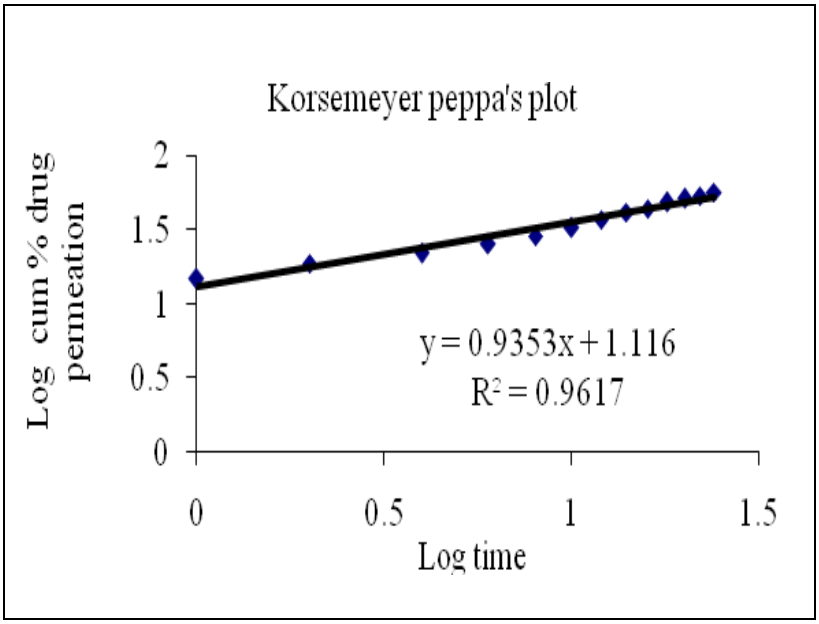


Table- 17 ***In vitro*** **Skin Permeation of control formulation- F_3 W (Without permeation enhancer)**

Time (hrs)	Cumulative % of Drug permeation			*Mean	Standard deviations (±SD)
	Trial I	Trial II	Trial III		
0	0	0	0	0	0.043437
0.25	0.56872	0.511848	0.597156	0.559242	0.044161
0.5	1.43128	1.37346	1.46019	1.421643	0.044885
0.75	2.592417	2.533649	2.621801	2.582622	0.436539
1	3.772512	3.200948	4.058294	3.677251	0.443779
2	5.255924	4.674882	5.546445	5.159084	0.451018
4	7.616114	7.025592	7.911374	7.517694	0.451018
6	10.74408	10.15355	11.03934	10.64566	0.465497
8	16.16114	15.55166	16.46588	16.05956	0.472736
10	19.54976	18.93081	19.85924	19.4466	0.479976
12	22.13744	21.509	22.45166	22.0327	0.487215
14	25.90047	25.26256	26.21943	25.79415	0.494455
16	28.8673	28.21991	29.191	28.7594	0.501694
18	31.59242	30.93555	31.92085	31.48294	0.508934
20	34.63981	33.97346	34.97299	34.52875	0.516173
22	37.16114	36.48531	37.49905	37.0485	0.523413
24	39.43128	38.74597	39.77393	39.31706	0.043437

*Average of three values

± Standard deviation

Fig 32: ***In vitro*** **skin permeation of formulation – F_3 W**

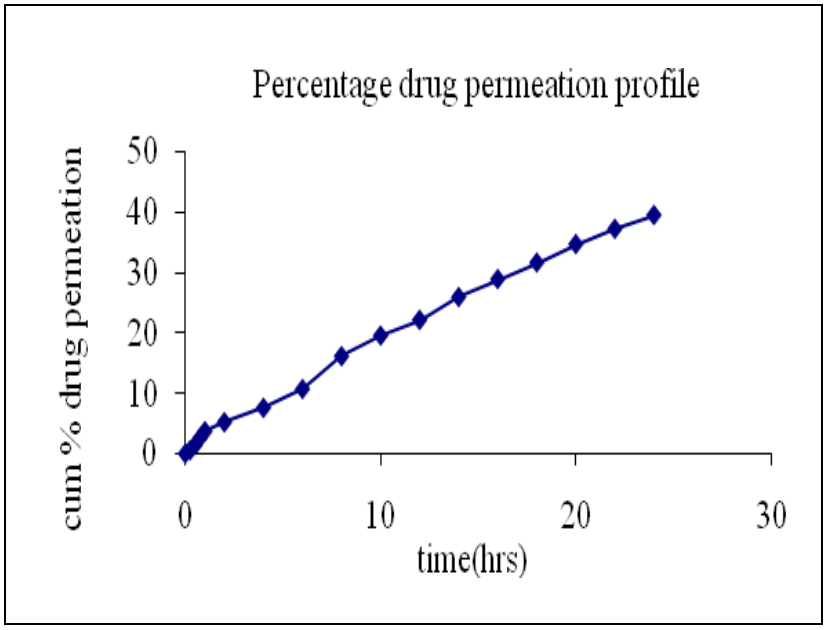


Fig 33**: Zero order permeation profile of formulation F_3 W**

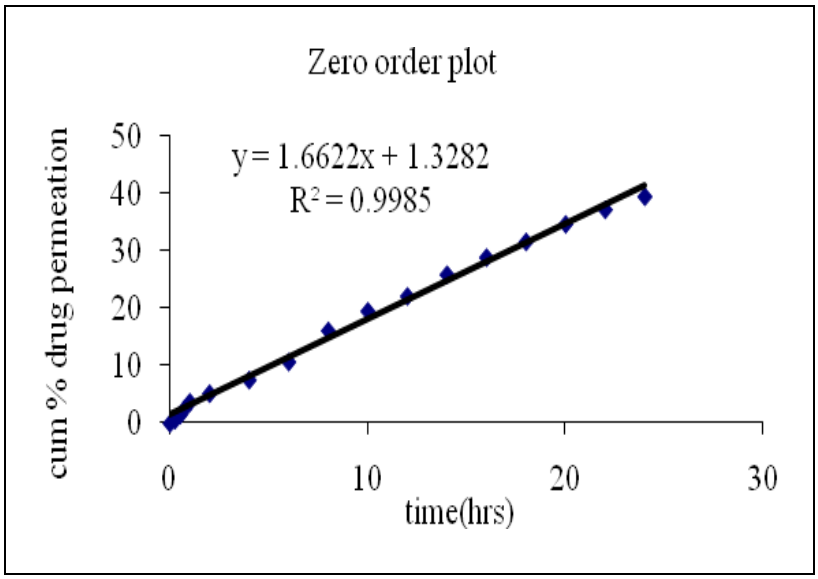


Fig 34: **First order permeation profile of formulation F_3 W**

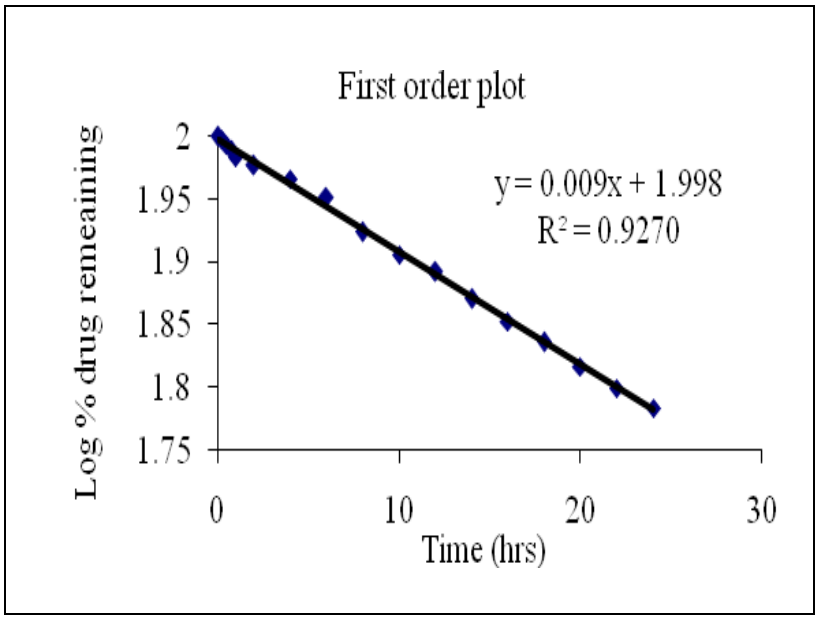


Fig 35: **Higuchi permeation profile of formulation F_3 W**

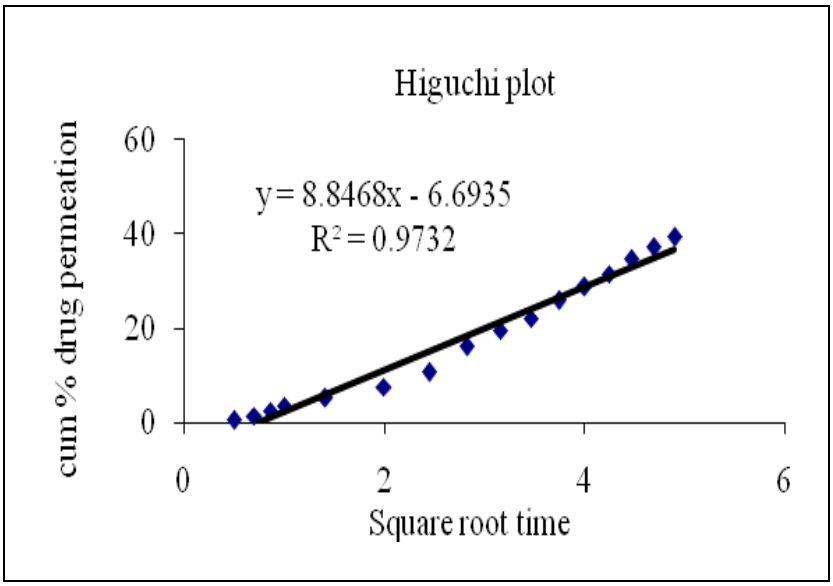


Fig 36: **Korsemeyer peppa's permeation profile of formulation F_3 W**

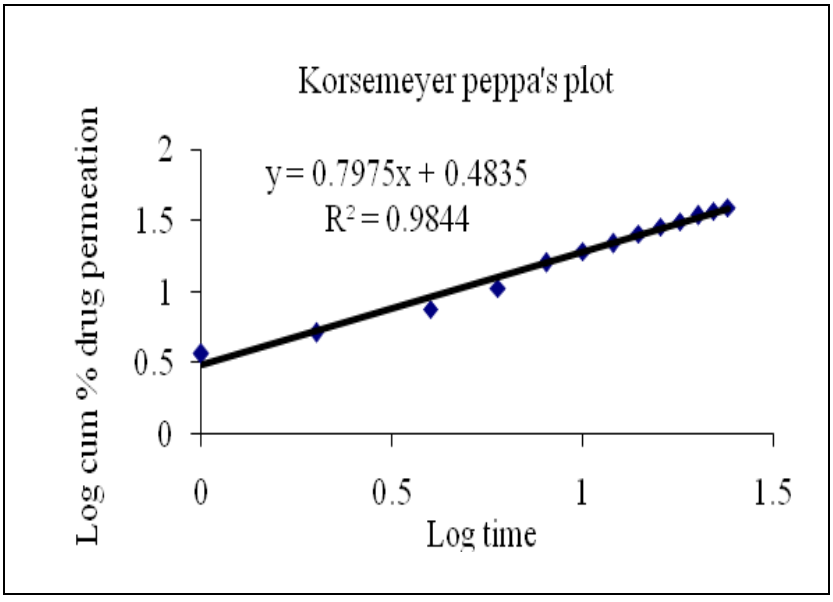


Table- 18 ***In vitro*** **Skin Permeation of control formulation- F_4 W (Without permeation enhancer)**

Time (hrs)	Cumulative % of Drug permeation			*Mean	Standard deviations (±SD)
	Trial I	Trial II	Trial III		
0	0	0	0	0	0
0.25	1.137441	1.080569	1.165877	1.127962	0.043437
0.5	2.578199	2.520379	2.607109	2.568562	0.044161
0.75	3.473934	2.903318	3.759242	3.378831	0.435815
1	4.952607	4.372512	5.242654	4.855924	0.443055
2	6.170616	5.581043	6.465403	6.072354	0.450294
4	8.260664	7.661611	8.56019	8.160821	0.457534
6	10.53555	9.936493	10.83507	10.4357	0.457534
8	13.11374	12.49573	13.42275	13.01074	0.472013
10	17.58768	16.96019	17.90142	17.4831	0.479252
12	21.27962	20.64265	21.5981	21.17346	0.486491
14	28.44076	27.79431	28.76398	28.33302	0.493731
16	31.16588	30.50995	31.49384	31.05656	0.50097
18	33.92891	33.26351	34.26161	33.81801	0.50821
20	38.15166	37.47678	38.4891	38.03918	0.515449
22	41.01422	40.32986	41.3564	40.90016	0.522689
24	46.47393	45.78009	46.82085	46.35829	0.529928

*Average of three values

± Standard deviation

Fig 37: ***In vitro*** **skin permeation of formulation – F_4 W**

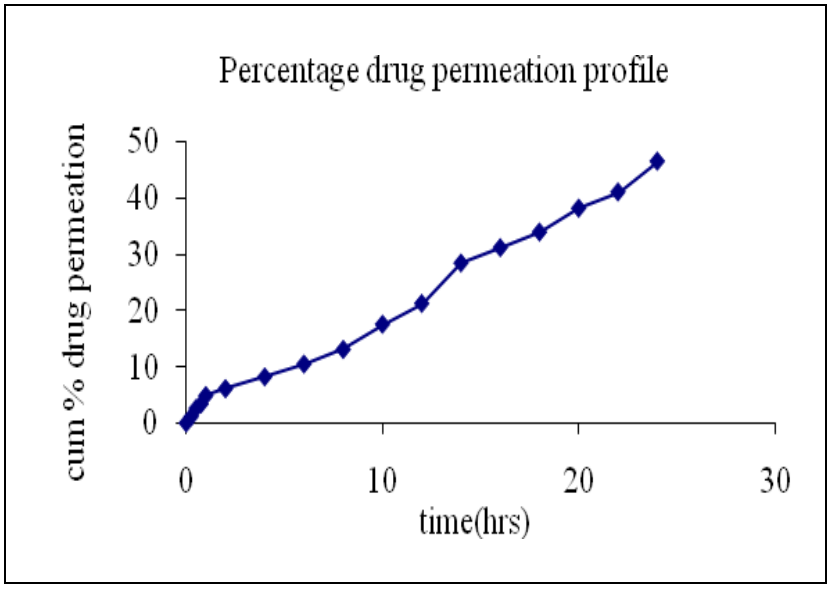


Fig 38: **Zero order permeation profile of formulation F_4 W**

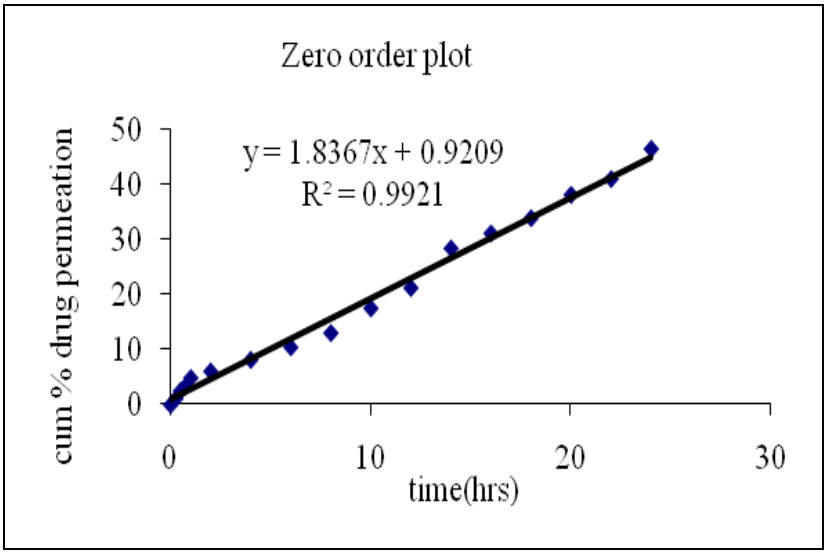


Fig 39: **First order permeation profile of formulation F_4 W**

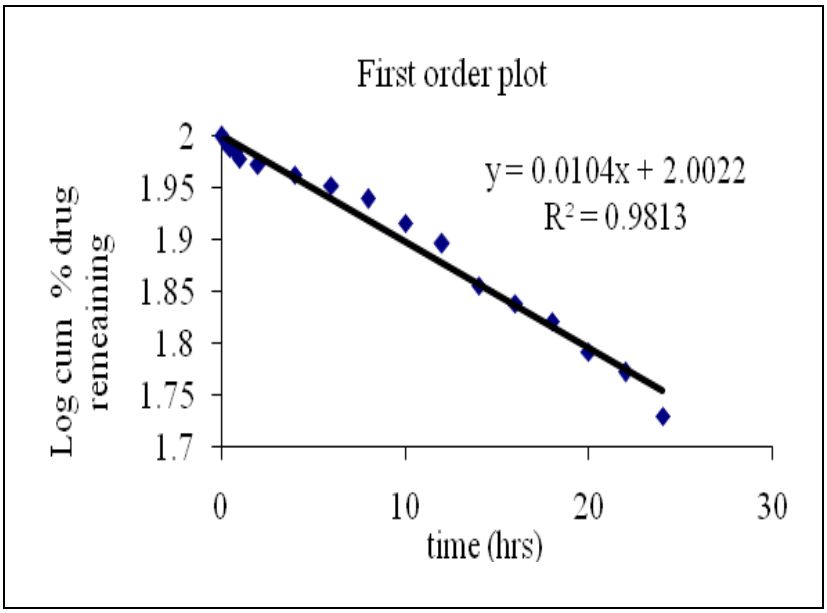


Fig 40: **Higuchi permeation profile of formulation** F_4 W

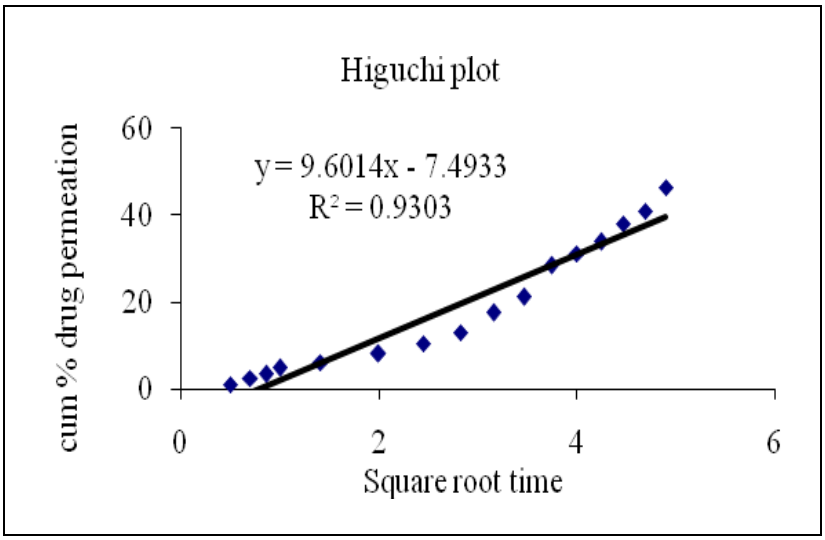


Fig 41: **Korsemeyer peppa's permeation profile of formulation F_4 W**

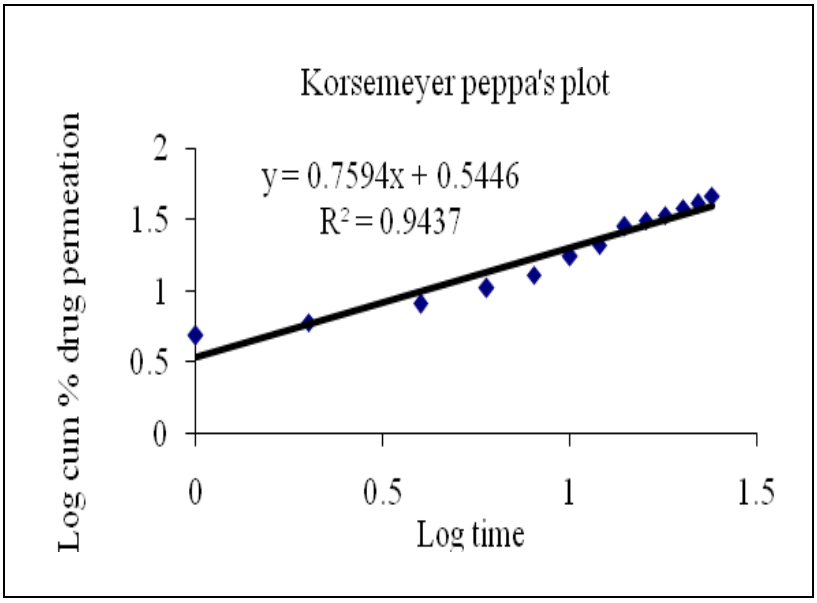


Table- 19 ***In-vitro*** **skin permeation of all formulations**

(Without permeation enhancer)

Time	Cumulative % of Drug Permeation			
	F_1 W	**F_2 W**	**F_3 W**	**F_4 W**
0	0	0	0	0
0.25	0.578199	3.033175	0.559242	1.127962
0.5	1.440916	7.917852	1.421643	2.568562
0.75	2.602212	11.4613	2.582622	3.378831
1	4.436493	14.77725	3.677251	4.855924
2	6.499684	18.71406	5.159084	6.072354
4	8.880411	21.8594	7.517694	8.160821
6	12.29273	25.27172	10.64566	10.4357
8	18.60869	28.5861	16.05956	13.01074
10	24.02733	32.44708	19.4466	17.4831
12	27.82512	36.93365	22.0327	21.17346
14	31.39542	41.48657	25.79415	28.33302
16	36.72401	43.83096	28.7594	31.05656
18	42.32275	48.76303	31.48294	33.81801
20	45.63555	51.20695	34.52875	38.03918
22	48.04471	53.96367	37.0485	40.90016
24	51.89447	56.75355	39.31706	46.35829

Fig 42**: *In vitro* skin permeation profile of various formulations**

(without permeation enhancer)

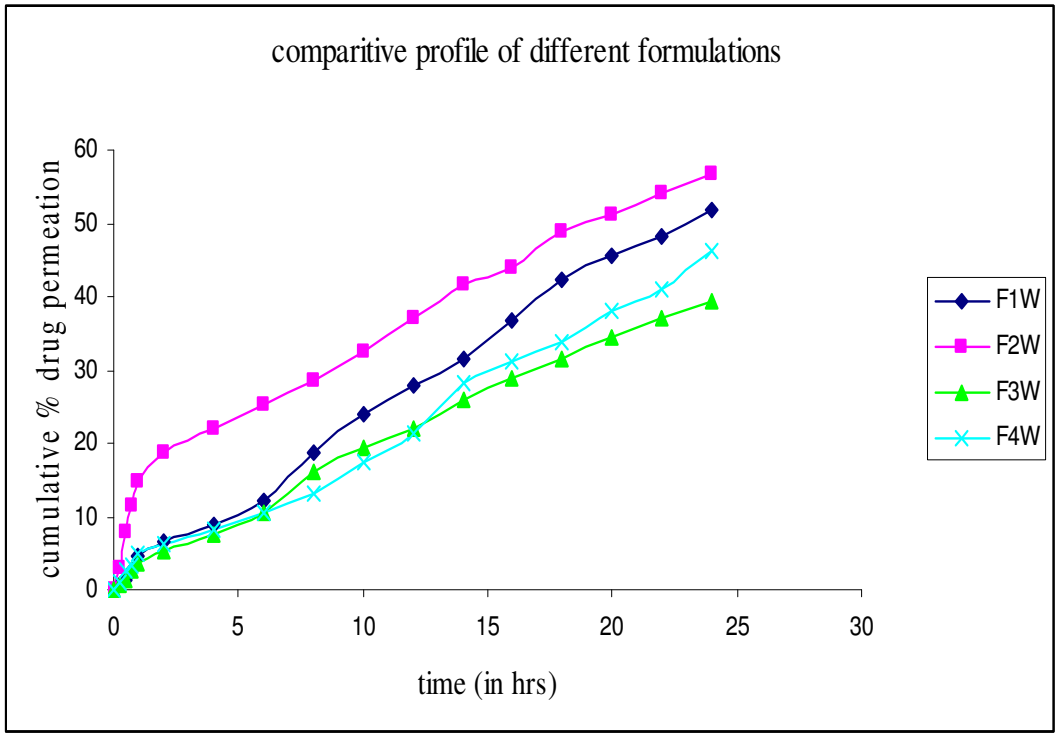


Table 20**: Cumulative Percentage And Kinetic Value Obtained From Different Formulations**

ormulation Code	Zero-order		First-order		Higuchi		Korse-Meyer Peppa's		Possible mechanism of drug release
	n	R^2	n	R^2	n	R^2	n	R^2	
F_1W	2.1976	0.9964	0.013	0.993	11.656	0.9603	0.8269	0.9768	zero-order, Non-fickian
F_2W	2.1354	0.9836	0.0138	0.955	11.189	0.985	0.9353	0.9617	zero-order, Non-fickian
F_3W	1.6622	0.9985	0.009	0.9270	8.8468	0.9732	0.7975	0.9844	zero-order, Non-fickian
F_4W	1.8367	0.9921	0.0104	0.9813	9.6014	0.9303	0.7590	0.9437	zero-order, Non-fickian

Table 21**:** ***In vitro*** **Skin Permeation of formulation-FPE_1**
(With permeation enhancer span 3%)

Time (hrs)	Cumulative % of Drug permeation			*Mean	Standard deviations (±SD)
	Trial I	Trial II	Trial III		
0	0	0	0	0	0
0.25	9.383886	8.530806	9.952607	9.2891	0.715624
0.5	11.24645	10.37915	11.82464	11.15008	0.727551
0.75	15.98104	15.09953	16.56872	15.8831	0.739478
1	18.80095	17.90521	19.3981	18.70142	0.751405
2	25.64455	24.7346	26.25118	25.54344	0.763332
4	34.87204	33.94787	35.48815	34.76935	0.775259
6	38.85308	37.92891	39.46919	38.75039	0.775259
8	43.16588	42.21327	43.80095	43.06003	0.799114
10	46.11848	45.43602	46.76303	46.10585	0.663597
12	54.79621	53.81991	55.45024	54.68878	0.820457
14	57.91943	56.92891	58.58294	57.81043	0.832385
16	63.92417	62.91943	64.59716	63.81359	0.844312
18	68.0237	67.00474	68.70616	67.91153	0.856239
20	74.16588	73.1327	74.85782	74.05213	0.868166
22	76.69668	75.64929	77.3981	76.58136	0.880093
24	80.95735	79.89573	81.66825	80.84044	0.89202

*Average of three values
± Standard deviations

Fig 43**: *In vitro* skin permeation of formulation – FPE_1**

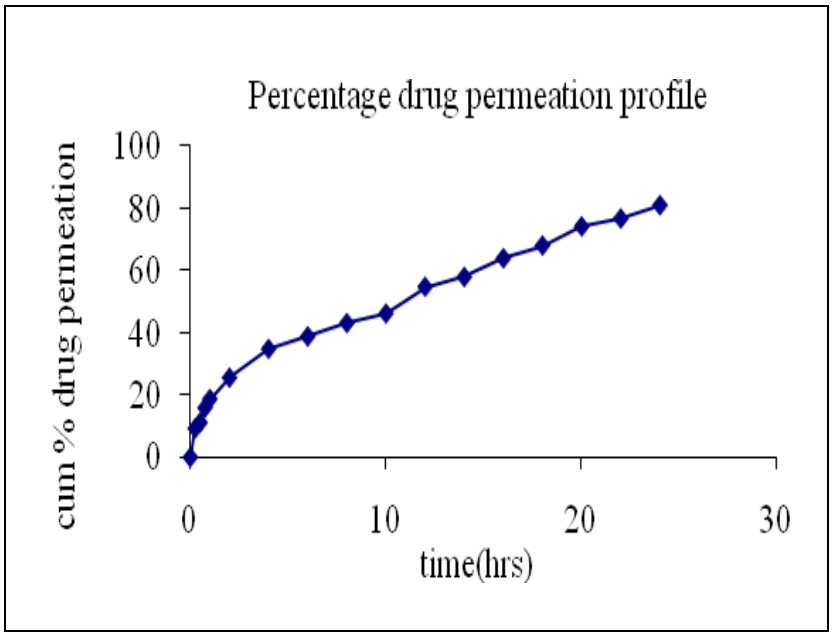


Fig 44**: Zero order permeation profile of formulation FPE_1**

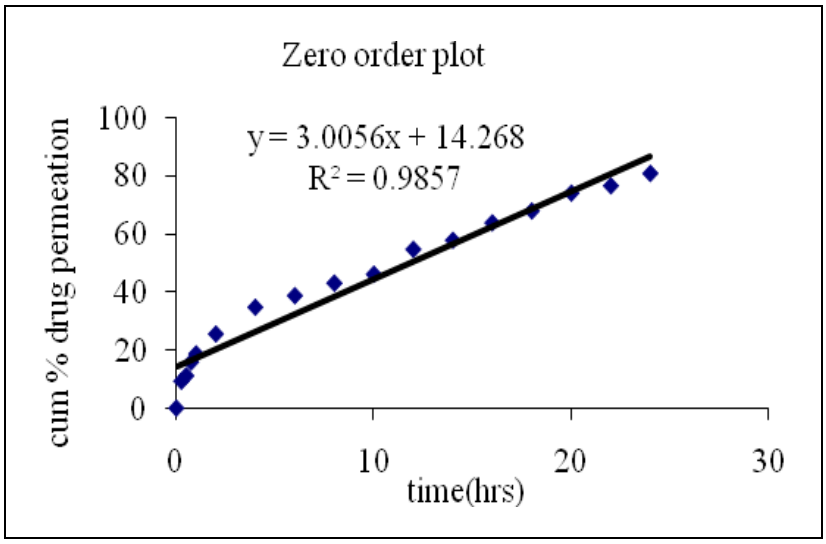


Fig 45**: First order permeation profile of formulation FPE_1**

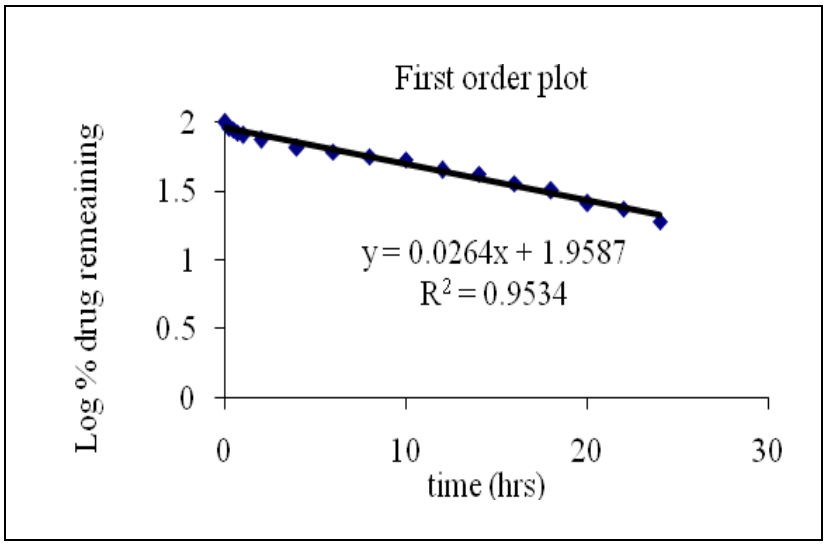


Fig 46**: Higuchi permeation profile of formulation FPE_1**

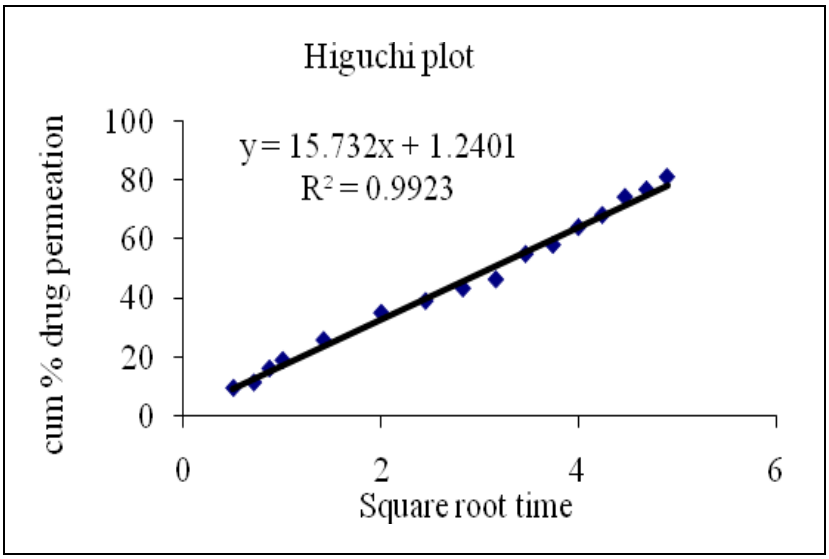


Fig 47**: Korsemeyer peppa's permeation profile of formulation FPE_1**

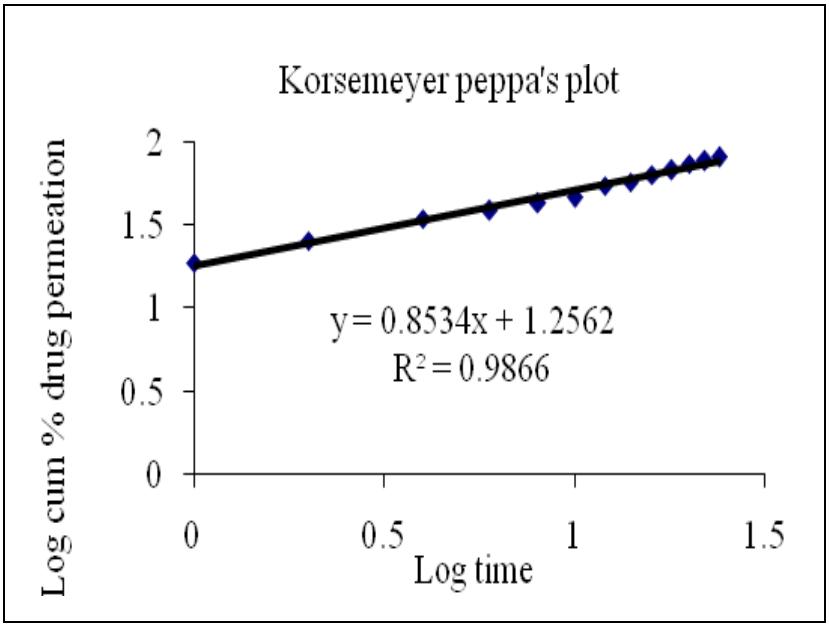


Table 22**: *In vitro* Skin Permeation of formulation- FPE_2 (With permeation enhancer span 3%)**

Time (hrs)	Cumulative % of Drug permeation			*Mean	Standard deviations (±SD)
	Trial I	Trial II	Trial III		
0	0	0	0	0	0
0.25	11.09005	10.23697	11.65877	10.99526	0.715624
0.5	12.98104	12.11374	13.55924	12.88468	0.727551
0.75	17.74408	16.86256	18.33175	17.64613	0.739478
1	20.59242	19.69668	21.18957	20.49289	0.751405
2	27.46445	26.5545	28.07109	27.36335	0.763332
4	36.72038	35.79621	37.33649	36.61769	0.775259
6	40.70142	39.77725	41.31754	40.59874	0.775259
8	45.07109	44.11848	45.70616	44.96524	0.799114
10	48.05213	47.08531	48.69668	47.94471	0.811041
12	56.75829	55.77725	57.41232	56.64929	0.822968
14	59.90995	58.91469	60.57346	59.79937	0.834895
16	65.94313	64.93365	66.61611	65.83096	0.846822
18	70.07109	69.04739	70.75355	69.95735	0.858749
20	76.24171	75.20379	76.93365	76.12638	0.870676
22	81.64455	80.59242	82.34597	81.52765	0.882603
24	88.82464	87.75829	89.53555	88.70616	0.89453

*Average of three values
± Standard deviations

Fig 48**: *In vitro* skin permeation of formulation – FPE_2**

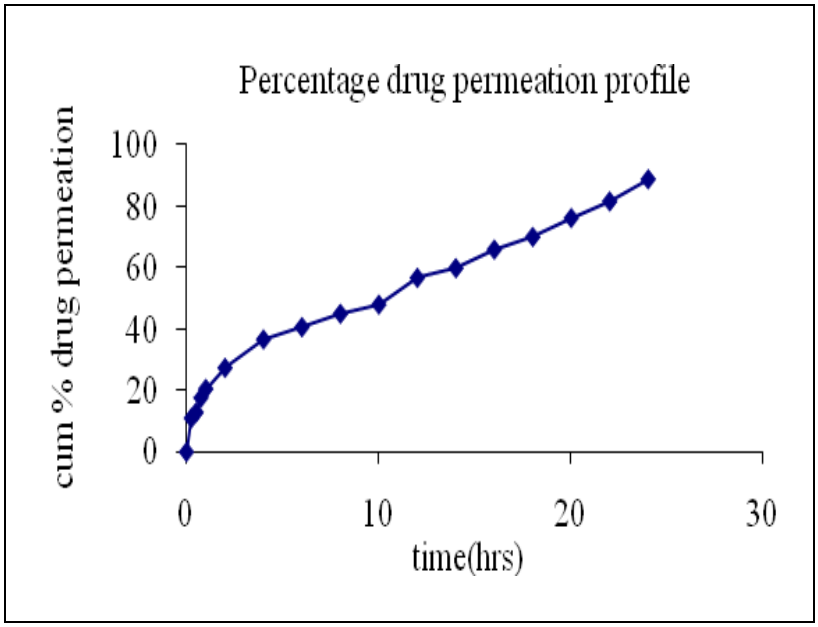


Fig 49**: Zero order permeation profile of formulation FPE$_2$**

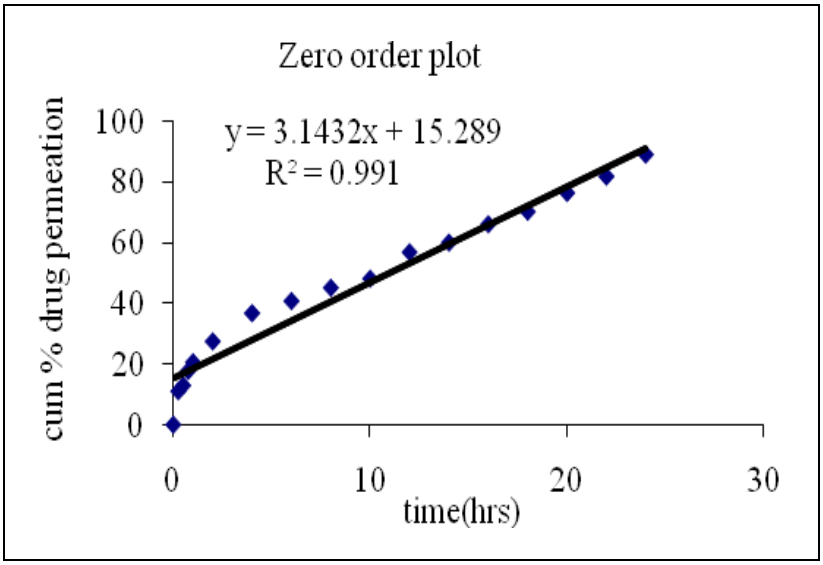


Fig 50**: First order permeation profile of formulation FPE$_2$**

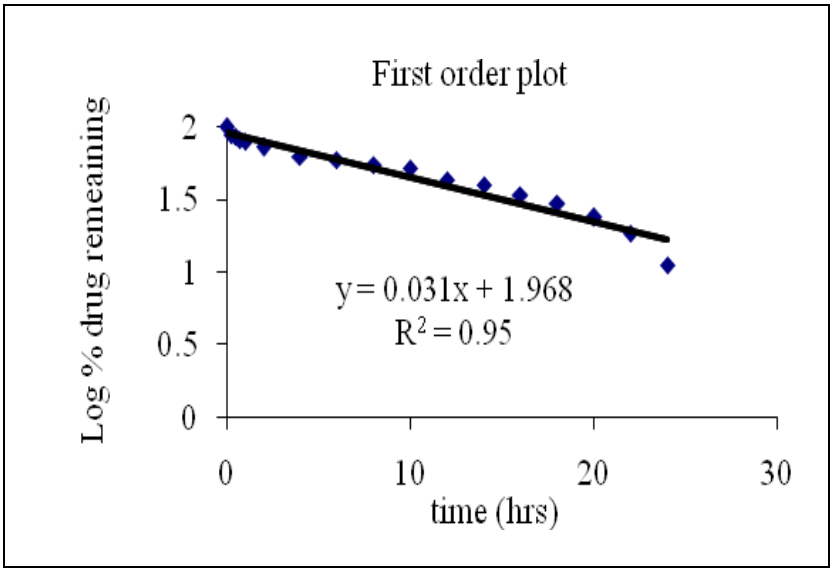


Fig 51**: Higuchi permeation profile of formulation FPE_2**

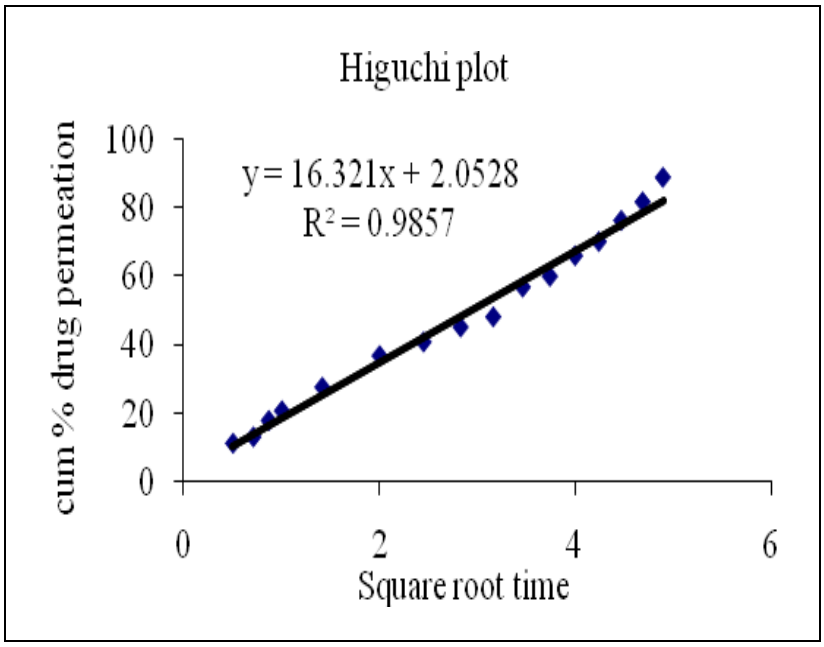


Fig 52**: Korsemeyer peppa's permeation profile of formulation FPE_2**

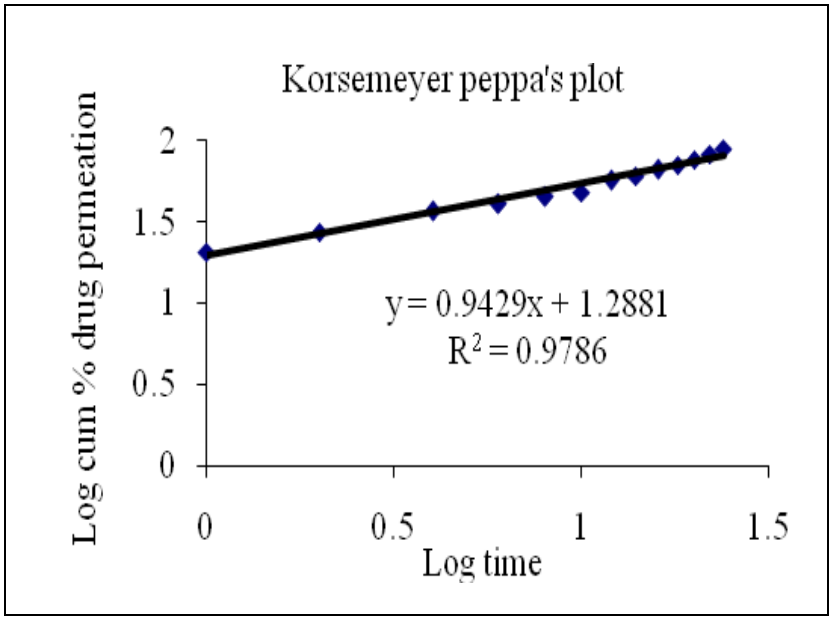


Table 23**: *In vitro* Skin Permeation of formulation- FPE_3**

(With permeation enhancer span 3%)

Time (hrs)	Cumulative % of Drug permeation			*Mean	Standard deviations (±SD)
	Trial I	Trial II	Trial III		
0	0	0	0	0	0
0.25	2.843602	1.990521	3.412322	2.748815	0.715624
0.5	5.450237	4.582938	6.028436	5.35387	0.727551
0.75	7.530806	6.649289	8.118483	7.432859	0.739478
1	11.35071	10.45498	11.94787	11.25118	0.751405
2	14.09479	13.18483	14.70142	13.99368	0.763332
4	17.7346	16.81043	18.35071	17.63191	0.775259
6	20.86256	19.93839	21.47867	20.75987	0.775259
8	26.31754	25.36493	26.95261	26.21169	0.799114
10	31.56872	30.6019	32.21327	31.4613	0.811041
12	33.77251	32.79147	34.42654	33.66351	0.822968
14	38.56398	37.56872	39.22749	38.4534	0.834895
16	41.15166	40.14218	41.82464	41.03949	0.846822
18	45.19431	44.17062	45.87678	45.08057	0.858749
20	50.71564	49.67773	51.40758	50.60032	0.870676
22	52.33649	51.28436	53.03791	52.21959	0.882603
24	57.38389	56.31754	58.09479	57.2654	0.89453

*Average of three values

± Standard deviations

Fig 53**: *In vitro* skin permeation of formulation – FPE_3**

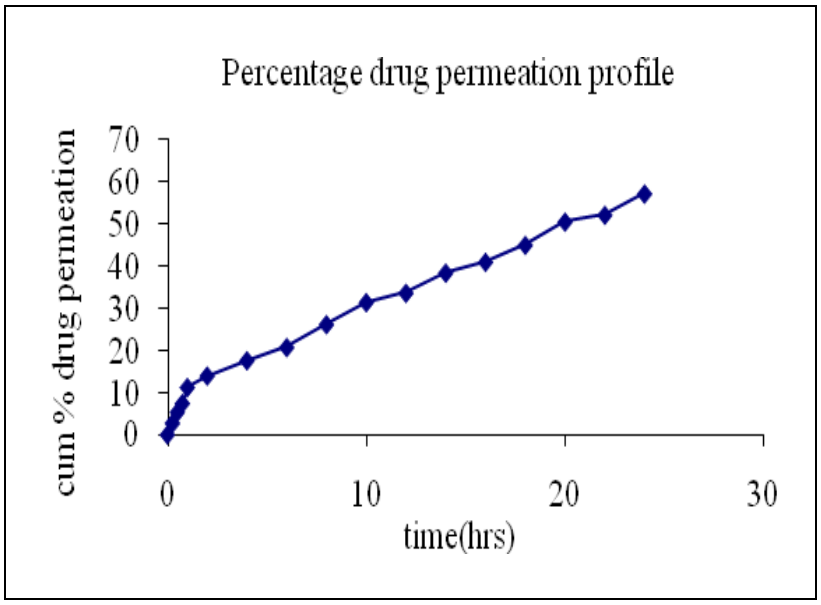


Fig 54**: Zero order permeation profile of formulation FPE_3**

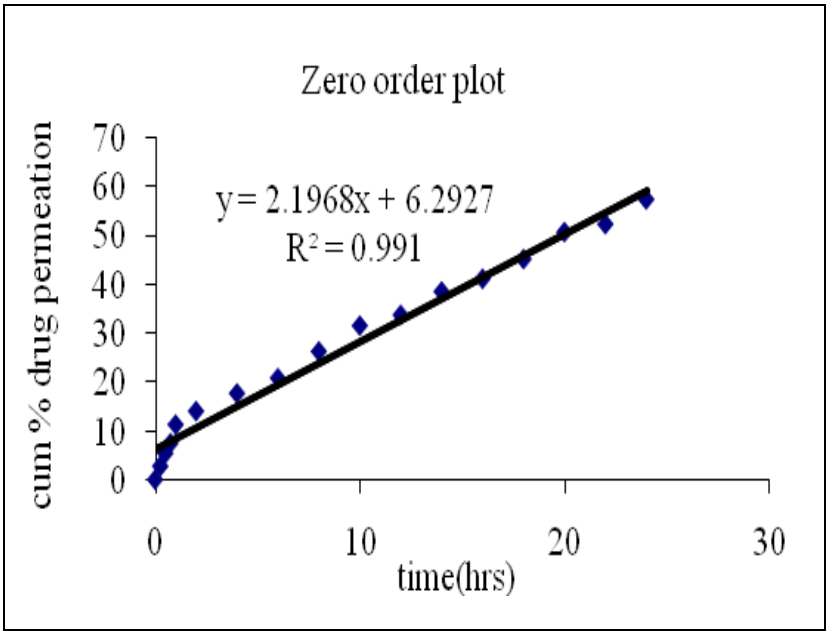


Fig 55: **First order permeation profile of formulation FPE_3**

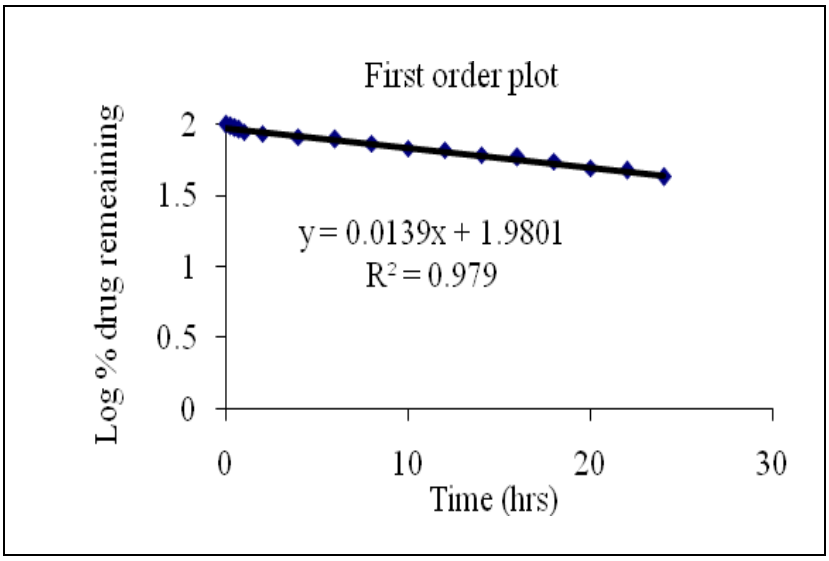


Fig 56: **Higuchi permeation profile of formulation FPE_3**

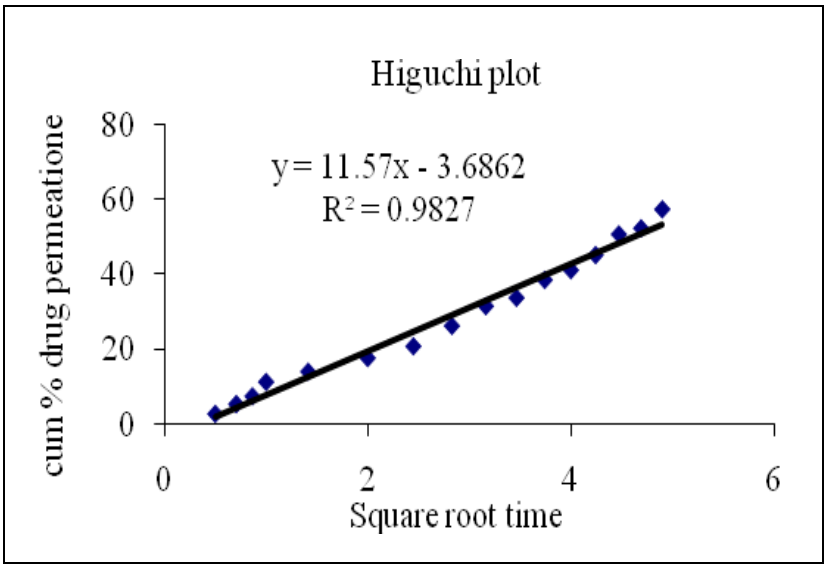


Fig 57: **Korsemeyer peppa's permeation profile of formulation FPE_3**

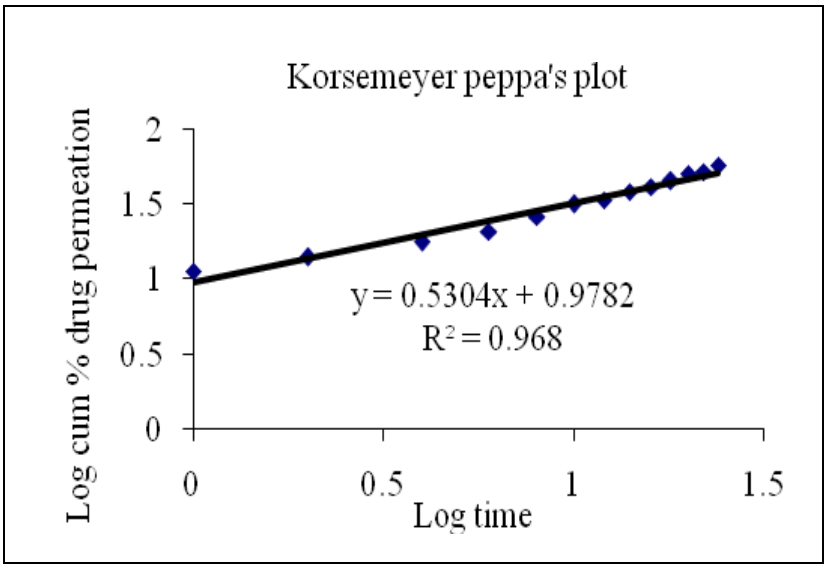


Table 24**: *In vitro* Skin Permeation of formulation- FPE_4**

(With permeation enhancer span 3%)

Time (hrs)	Cumulative % of Drug permeation			*Mean	Standard deviations (±SD)
	Trial I	Trial II	Trial III		
0	0	0	0	0	0
0.25	3.981043	3.127962	4.549763	3.886256	0.715624
0.5	6.322275	5.454976	6.900474	6.225908	0.727551
0.75	8.985782	8.104265	9.57346	8.887836	0.739478
1	12.54502	11.64929	13.14218	12.4455	0.751405
2	14.73934	13.82938	15.34597	14.63823	0.763332
4	18.95735	18.03318	19.57346	18.85466	0.775259
6	21.51659	20.59242	22.1327	21.4139	0.775259
8	27.28436	26.33175	27.91943	27.17852	0.799114
10	32.83412	31.8673	33.47867	32.7267	0.811041
12	35.05687	34.07583	35.7109	34.94787	0.822968
14	39.8673	38.87204	40.53081	39.75671	0.834895
16	42.47393	41.46445	43.14692	42.36177	0.846822
18	51.08531	50.06161	51.76777	50.97156	0.858749
20	56.98578	55.94787	57.67773	56.87046	0.870676
22	62.40284	61.35071	63.10427	62.28594	0.882603
24	65.90521	64.83886	66.61611	65.78673	0.89453

*Average of three values
± Standard deviations

Fig 58: ***In vitro*** **skin permeation of formulation – FPE_4**

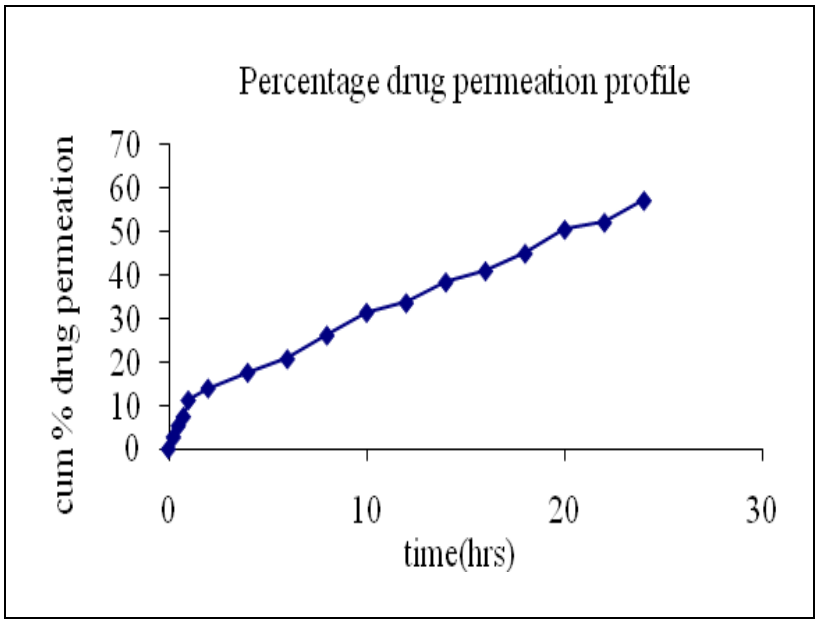


Fig 59: **Zero order permeation profile of formulation FPE_4**

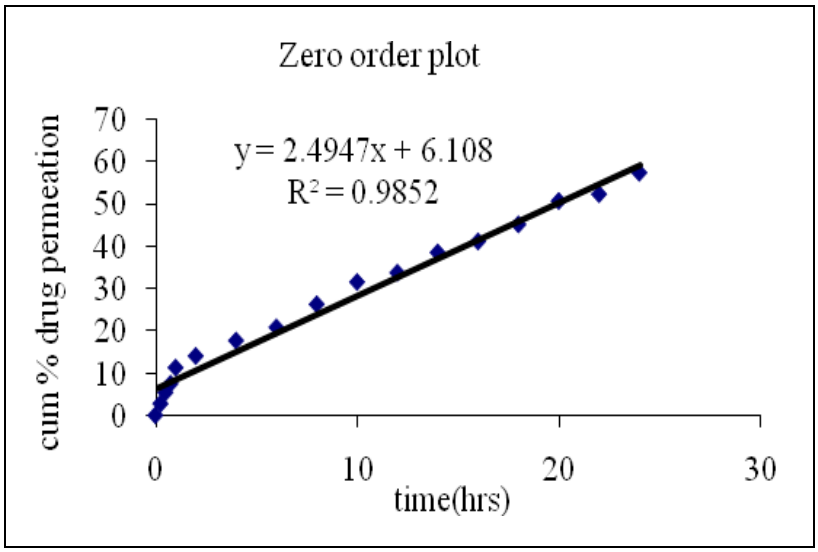


Fig 60: **First order permeation profile of formulation FPE_4**

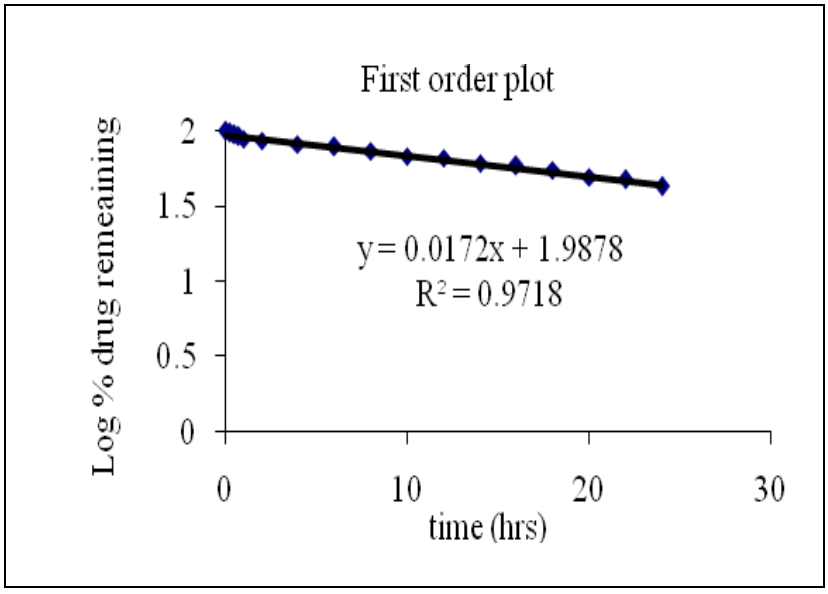


Fig 61: **Higuchi permeation profile of formulation FPE_4**

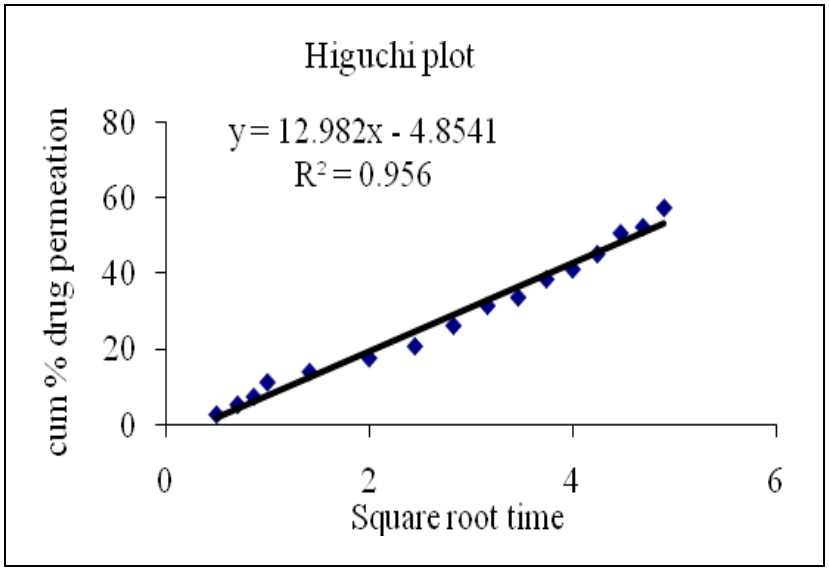


Fig 62: **Korsemeyer peppa's permeation profile of formulation FPE_4**

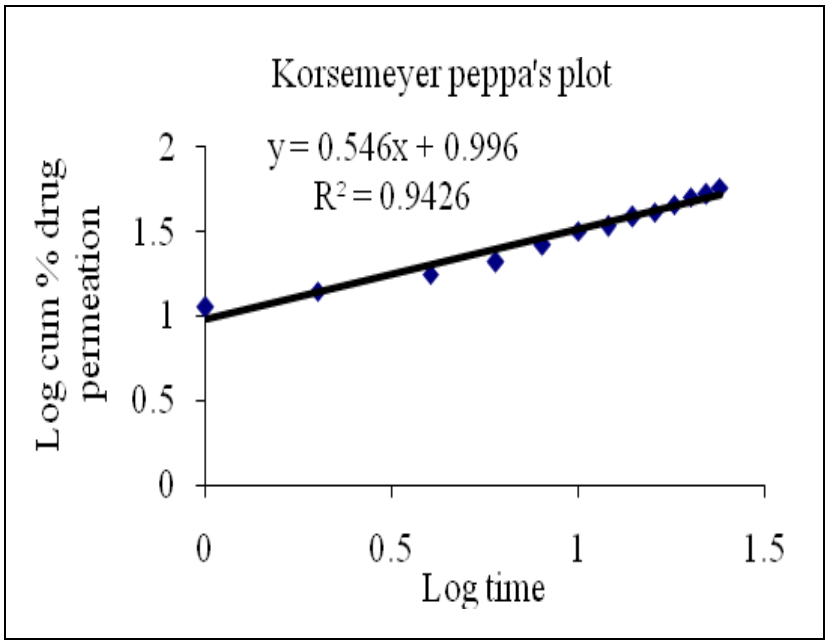


Table- 25 ***In-vitro*** **skin permeation of all formulations**

(With permeation enhancer)

Time	Cumulative % of Drug Permeation			
	FPE_1	**FPE_2**	**FPE_3**	**FPE_4**
0	0	0	0	0
0.25	9.2891	10.99526	2.748815	3.886256
0.5	11.15008	12.88468	5.35387	6.225908
0.75	15.8831	17.64613	7.432859	8.887836
1	18.70142	20.49289	11.25118	12.4455
2	25.54344	27.36335	13.99368	14.63823
4	34.76935	36.61769	17.63191	18.85466
6	38.75039	40.59874	20.75987	21.4139
8	43.06003	44.96524	26.21169	27.17852
10	46.10585	47.94471	31.4613	32.7267
12	54.68878	56.64929	33.66351	34.94787
14	57.81043	59.79937	38.4534	39.75671
16	63.81359	65.83096	41.03949	42.36177
18	67.91153	69.95735	45.08057	50.97156
20	74.05213	76.12638	50.60032	56.87046
22	76.58136	81.52765	52.21959	62.28594
24	80.84044	88.70616	57.2654	65.78673

Fig 63**: *In vitro* skin permeation profile of various formulations**

(with permeation enhancer)

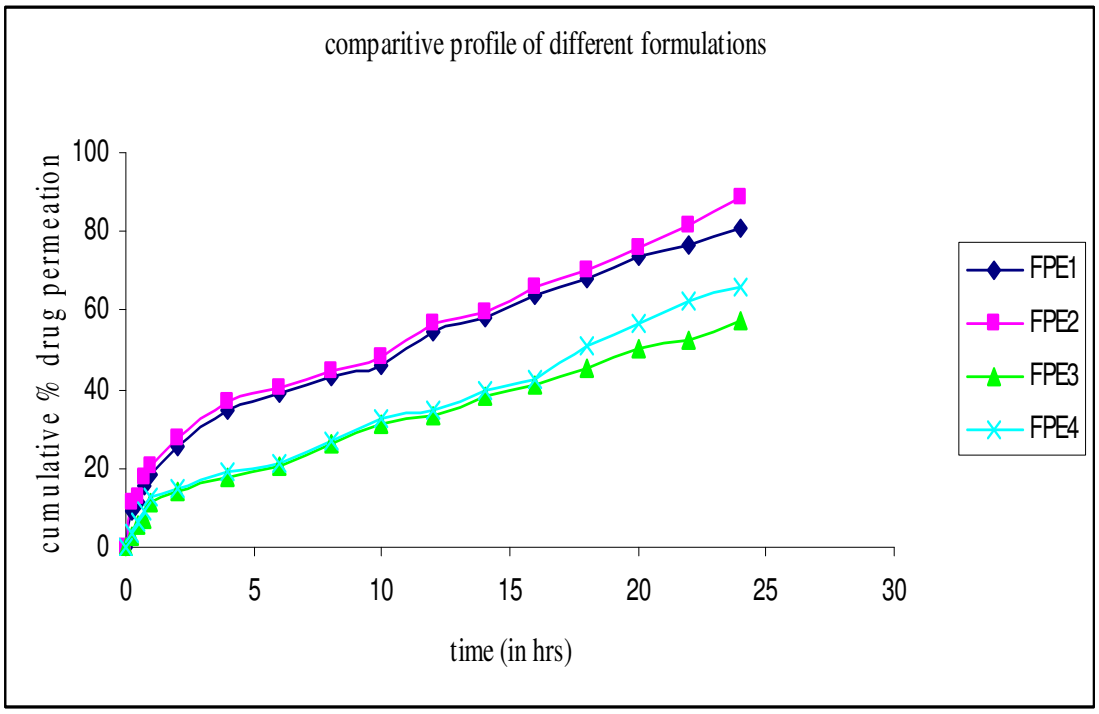


Table 26: **Cumulative Percentage And Kinetic Value Obtained From Different Formulations**

Formulation Code	Zero-order		First-order		Higuchi		Korse-Meyer Peppa's		Possible mechanism of drug release
	n	R^2	n	R^2	n	R^2	n	R^2	
FPE_1	3.0056	0.9857	0.0264	0.9534	15.732	0.9923	0.8534	0.9866	zero-order, Non-fickian
FPE_2	3.1432	0.9910	0.0310	0.950	16.231	0.9857	0.9429	0.9786	zero-order, Non-fickian
FPE_3	2.1968	0.991	0.0139	0.979	11.57	0.9827	0.5304	0.968	zero-order, Non-fickian
FPE_4	2.4947	0.9852	0.0172	0.9718	12.982	0.956	0.546	0.9426	zero-order, Non-fickian

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The *in vitro* skin permeation kinetics:

Table-27**: The *in vitro* skin permeation kinetic data for transdermal patches (Without permeation enhancer)**

S. No	Formulation Code	Slope of zero order plot	Permeation coefficient (P)	Flux (J)
1	F_1 W	2.1354	3.40	17
2	F_2 W	2.1976	3.49	17.45
3	F_3 W	1.6622	2.64	13.2
4	F_4 W	1.8362	2.92	14.6

The *in vitro* skin permeation kinetics:

Table-28**: The *in vitro* skin permeation kinetic data for transdermal patches (With permeation enhancer)**

S. No	Formulation Code	Slope of zero order plot	Permeation coefficient (P)	Flux (J)	Enhancement ratio (E_r)
1	FPE_1	3.005	4.78	23.9	1.405
2	FPE_2	3.143	5.00	25	1.432
3	FPE_3	2.196	3.49	17.45	1.321
4	FPE_4	2.494	3.97	19.85	1.359

Skin irritation test

The visual score was 0 (none) on both the erythema scale and the edema scale. This indicates there was no sign of skin reaction. Hence, the fabricated transdermal patch was suitable for further studies.

Statistical analysis by ANOVA

The statistical analysis of the cumulative percentage of drug permeation data showed that the formulations are having 'p' value < 0.0001. This result suggests that the prepared Transdermal patches are extremely significant for the approach

IN-VIVO STUDIES FOR THE BEST FORMULATION (FPE_2)

HPLC analytical data for the plasma and control samples.

Table- 29

S. No	Sample	Area under curve (AUC)					
		Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**
1	Control	1729250.73	1729330.15	1729300.93	1729200.69	1729190.39	1729230.58
S. No	**Sample Time (in hrs)**	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**
2	0.5	1429283.25	1429363.54	1429333.19	1429233.70	1429223.92	1429263.66
3	2	2914583.07	2914563.11	2914523.29	2914533.57	2914633.72	2914663.85
4	4	3787204.87	3787154.19	3787284.74	3787144.57	3787254.48	3787184.30
5	8	5039821.69	5039761.07	5039871.39	5039841.55	5039901.17	5039771.91
6	16	5785842.70	5785922.03	5785792.22	5785822.64	5785892.91	5785782.44
7	24	6874342.48	6874392.12	6874322.60	6874422.55	6874282.28	6874292.88

In-vivo drug absorption kinetics

Table- 30

S. No	Time(in hrs)	Mean Area under curve (AUC)	Concentration (µg/ml)	% drug absorption (%)
1	control	1729250.73	10	--
2	0.5	1429283.25	8.265	16.54
3	2	2914583.07	16.85	33.71
4	4	3787204.87	21.90	43.80
5	8	5039821.69	29.14	58.28
6	16	5785842.70	33.45	66.90
7	24	6874342.48	39.75	79.50

In-vivo absorption studies

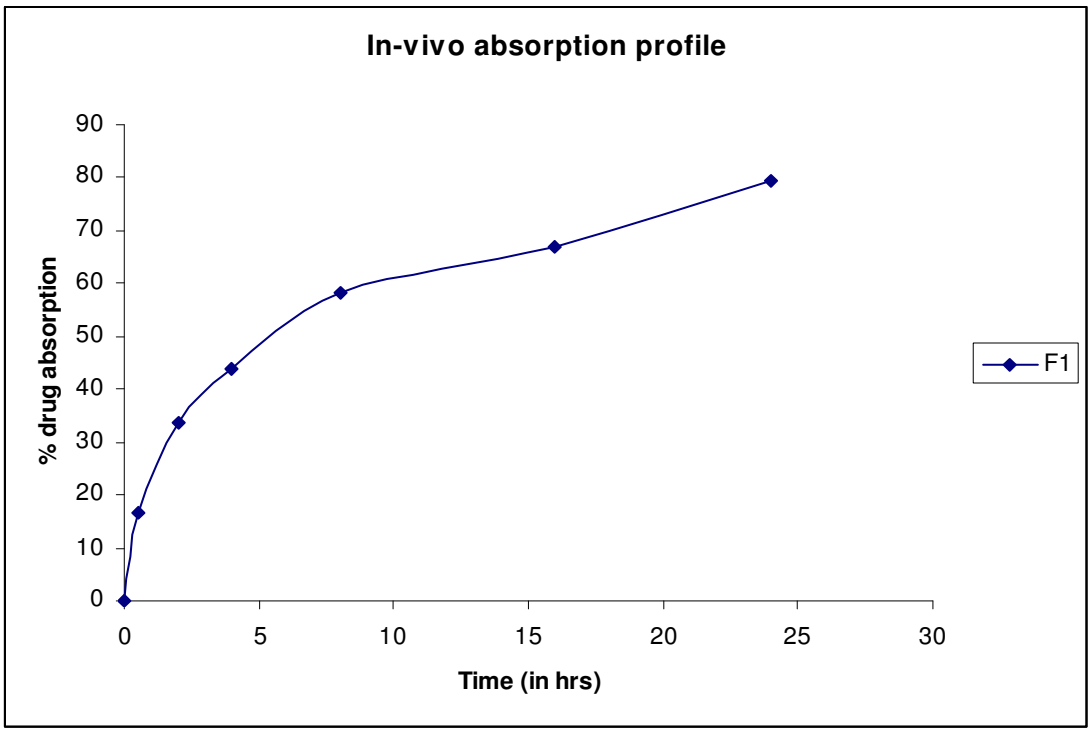

Fig: 64 ***In-vivo*** **absorption curve**

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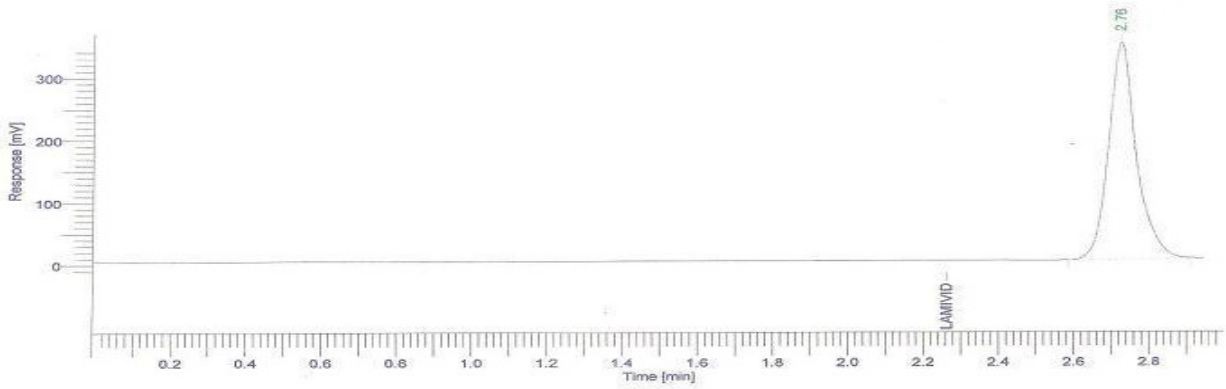
Cycle	: 1		

Result File :
Sequence File : D:\Sequence\PAVAN LAMIVIDINE1.seq



SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Analysis: test mix HPLC

Peak #	Component Name	RT [min]	AUC [uV*sec]	Height [uV]	Area [%]	concentration Amount
1	LAMIVIDINE	2.755	1729250.73	340844.91	100.00	10.0000
			1729250.73	340844.91	100.00	10.0000

Analysed BY :

Date :

Fig: 65 **HPLC analysis of standard solution of Lamivudine**

Software Version	: 6.3.1.0504	Date	: 1/12/2010 11:38:59 AM
Sample Name	: LAMIVIDINE	Data Acquisition Time	: 1/12/2010 11:25:21 AM
Instrument Name	: PerkinElmer HPLC	Channel	: A
Rack/Vial	: 0/0	Operator	: manager
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 2		

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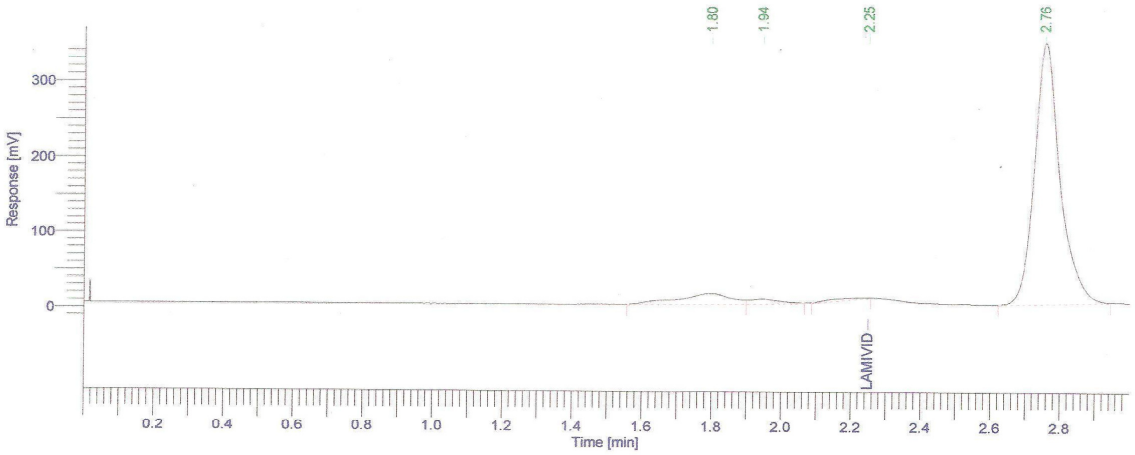
Sequence File : D:\Sequence\pavan lamividine sequence.seq



SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Analysis: test mix HPLC

Peak #	Component Name	RT [min]	AUC [uV*sec]	Height [uV]	Area [%]	concentration Amount
1		1.798	132988.69	13196.45	5.86	0.1330
2		1.945	29330.42	5481.46	1.29	0.0293
3	LAMIVIDINE	2.249	13567.35	330.47	0.60	0.1235
4		2.755	1429283.25	340844.91	76.20	1.7293
5		3.739	364185.05	56493.44	16.05	0.3642
			1969354.76	416346.73	100.00	2.3793

Analysed BY :

Fig: 66 **HPLC analysis of rat plasma after 0.5hrs**

Software Version	: 6.3.1.0504	Date	: 1/12/2010 11:05:23 AM
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Instrument Name	: PerkinElmer HPLC	Channel	: A
Rack/Vial	: 0/0	Operator	: manager
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 3		

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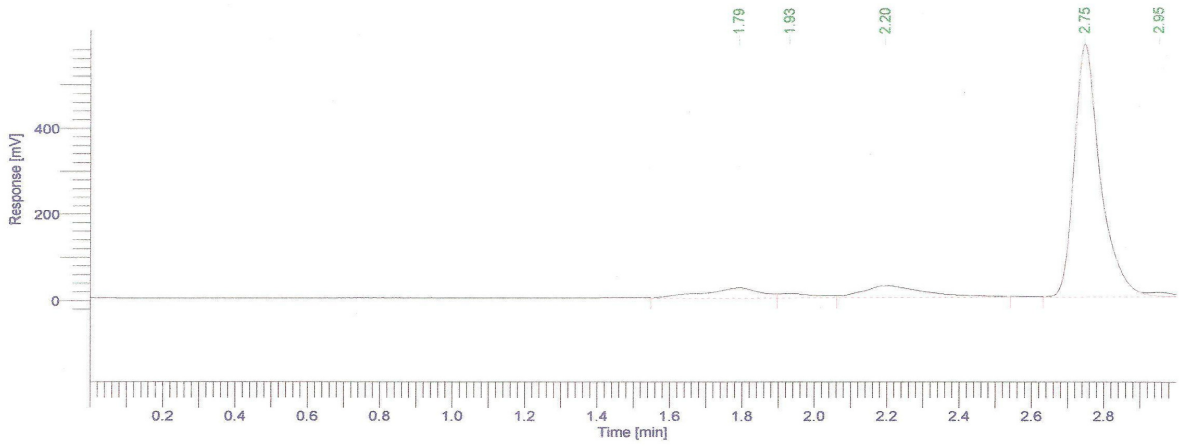
Sequence File : D:\Sequence\pavan lamividine sequence.seq



SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Analysis: test mix HPLC

Peak #	Component Name	RT [min]	AUC [uV*sec]	Height [uV]	Area [%]	concentration Amount
1	LAMIVIDINE	1.792	227823.70	21644.42	6.46	0.2278
2		1.931	57117.54	9254.36	1.62	0.0571
3		2.195	286845.46	25607.16	8.14	0.2868
4		2.747	2914583.07	579669.52	82.68	2.9146
5		2.952	38885.87	7087.86	1.10	0.0389
			3525255.63	643263.31	100.00	3.5253

Analysed BY :

Fig: 67 **HPLC analysis of rat plasma after 2 hrs**

Software Version	: 6.3.1.0504	Date	: 1/12/2010 11:21:04 AM
Sample Name	: LAMIVIDINE	Data Acquisition Time	: 1/12/2010 11:07:32 AM
Instrument Name	: PerkinElmer HPLC	Channel	: A
Rack/Vial	: 0/0	Operator	: manager
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 4		

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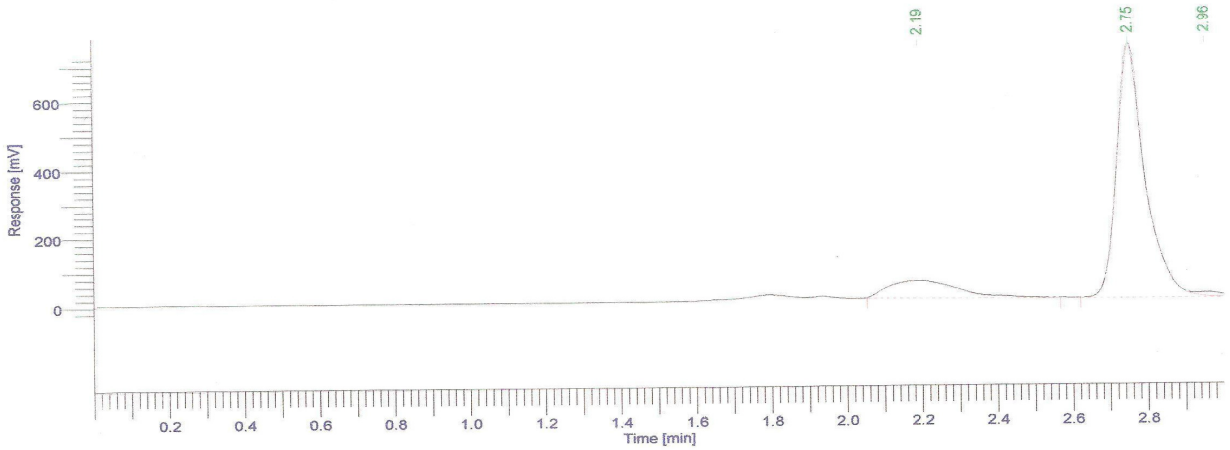
Sequence File : D:\Sequence\pavan lamividine sequence.seq



SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Analysis: test mix HPLC

Peak #	Component Name	RT [min]	AUC [uV*sec]	Height [uV]	Area [%]	concentration Amount
1	LAMIVIDINE	2.193	603371.94	48533.97	10.64	0.6034
2		2.752	3787204.87	728538.38	66.78	3.7872
3		2.955	54058.14	8491.98	0.95	0.0541
4		3.214	21070.41	3750.51	0.37	0.0211
5		3.719	1205374.44	176199.95	21.25	1.2054
			5671079.82	965514.78	100.00	5.6711

Analysed BY :

Fig: 68 **HPLC analysis of rat plasma after 4 hrs**

Software Version	: 6.3.1.0504	Date	: 1/12/2010 11:43:00 AM
Sample Name	: LAMIVIDINE	Data Acquisition Time	: 1/12/2010 11:27:48 AM
Instrument Name	: PerkinElmer HPLC	Channel	: A
Rack/Vial	: 0/0	Operator	: manager
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 5		

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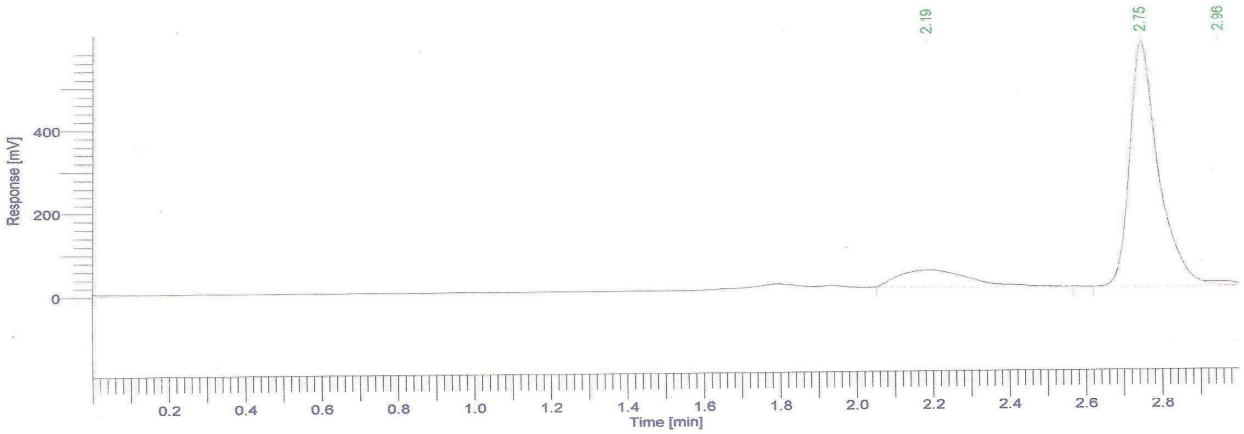
Sequence File : D:\Sequence\pavan lamividine sequence.seq



SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Analysis: test mix HPLC

Peak #	Component Name	RT [min]	AUC [uV*sec]	Height [uV]	Area [%]	concentration Amount
1	LAMIVIDINE	2.193	603371.94	48533.97	6.46	0.6034
2		2.752	5039821.69	579669.52	82.68	4.6350
3		2.955	54058.14	8491.98	8.14	0.0541
4		3.214	21070.41	3750.51	1.62	0.0211
5		3.719	1205374.44	176199.95	1.10	1.2054
			6923696.62	816645.93	100.00	6.5190

Analysed BY :

Fig: 69 **HPLC analysis of rat plasma after 8hrs**

Software Version	: 6.3.1.0504	Date	: 1/12/2010 12:02:09 PM
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Instrument Name	: PerkinElmer HPLC	Channel	: A
Rack/Vial	: 0/0	Operator	: manager
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 6		

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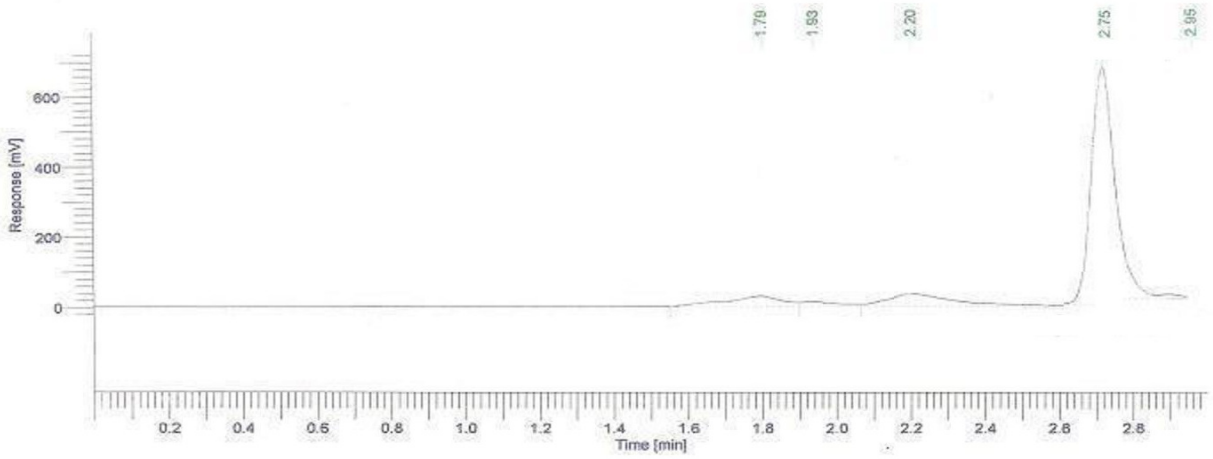
Sequence File : D:\Sequence\pavan lamividine sequence.seq



SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Analysis: test mix HPLC

Peak #	Component Name	RT [min]	AUC [uV*sec]	Height [uV]	Area [%]	concentration Amount
1	LAMIVIDINE	1.792	227823.70	21644.42	17.54	0.7823
2		1.931	57117.54	9254.36	5.87	0.1951
3		2.195	286845.46	25607.16	7.54	0.0087
4		2.747	5785842.70	682338.54	64.00	5.8707
5		2.952	38885.87	7087.86	15.05	0.0389
			7485015.05	792132.18	100.00	6.8957

Analysed BY :

Fig: 70 **HPLC analysis of rat plasma after 16 hrs**

Software Version	: 6.3.1.0504	Date	: 1/12/2010 12:16:09 PM
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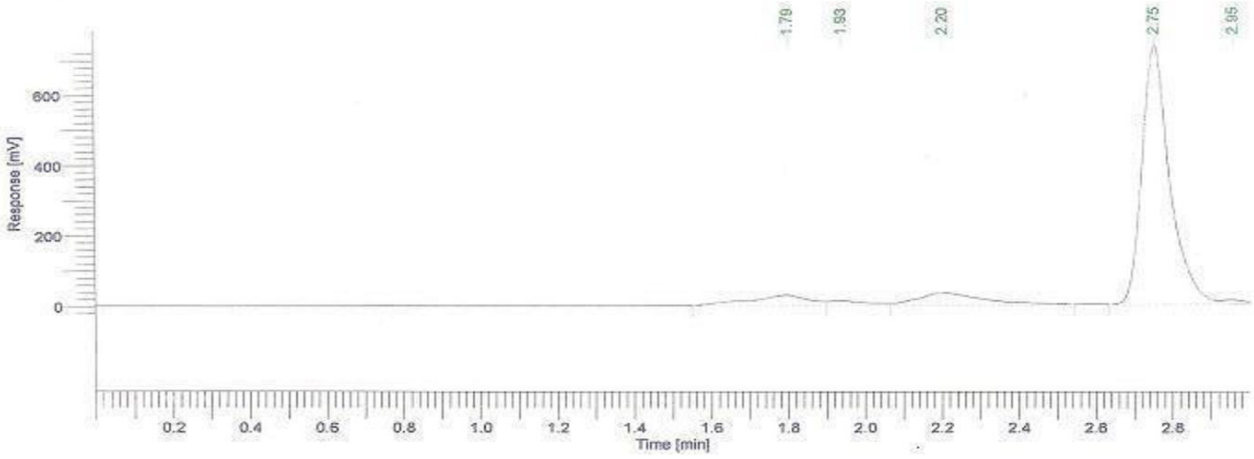
Rack/Vial	: 0/0	Operator	: manager
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 7		

Result File : D:\Data\lamividine sequence002.rst
Sequence File : D:\Sequence\pavan lamividine sequence.seq



SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

Analysis: test mix HPLC

Peak #	Component Name	RT [min]	AUC [uV*sec]	Height [uV]	Area [%]	concentration Amount
1	LAMIVIDINE	1.792	227823.70	21644.42	10.64	0.2278
2		1.931	57117.54	9254.36	0.37	0.0571
3		2.195	286845.46	25607.16	0.95	0.2868
4		2.747	6874342.48	728538.38	66.78	6.9607
5		2.952	38885.87	7087.86	21.25	0.0389
			7485015.05	792132.18	100.00	7.5713

Analysed BY :

Fig: 71 **HPLC analysis of rat plasma after 24 hrs**

INVITRO/INVIVO CORRELATION:

Table- 31

S.No	Time (in hrs)	*In-vitro* skin permeation (%)	*In-vivo* absorption (%)
1.	0.5	12.88	16.54
2.	2	27.36	33.71
3.	4	36.61	43.80
4.	8	44.96	58.28
5.	16	65.83	66.90
6.	24	88.70	79.50

By establishing a relationship, between the *in vitro* permeation and the *in vivo* absorption parameters.

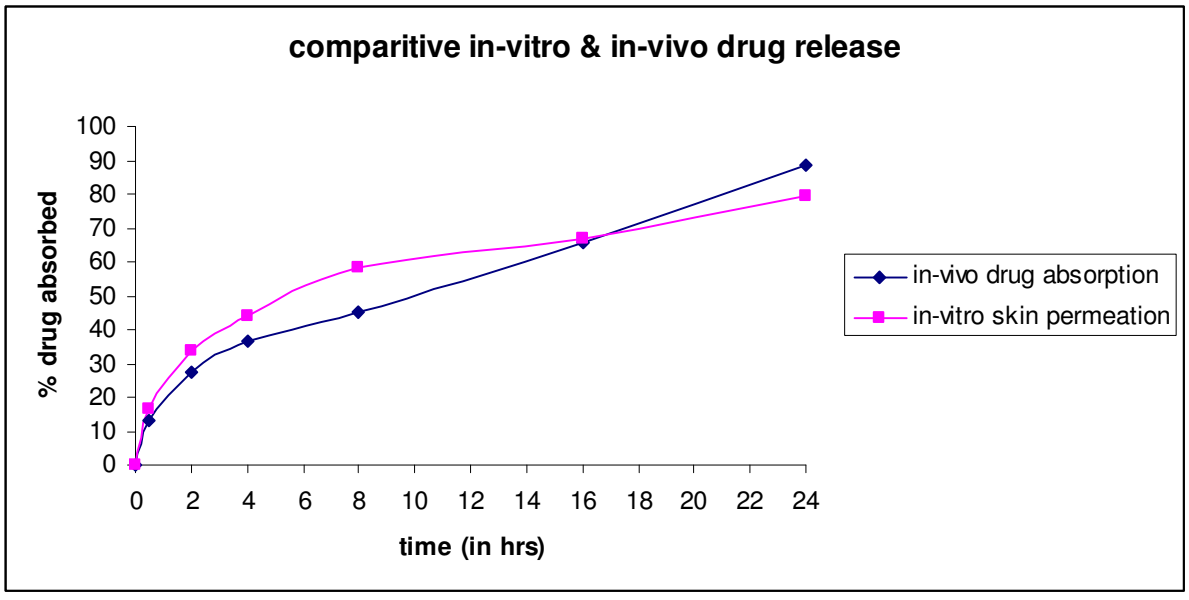


Fig: 72 **Comparitive curve for *Invitro & Invivo* studies**.

Table- 32 **Comparison of *Invitro* and *Invivo* studies**

***In-vitro* skin permeation (%)**	***In-vivo* absorption (%)**
12.88	16.54
27.36	33.71
36.61	43.80
44.96	58.28
65.83	66.90
88.70	79.50

Slope- 0.814983

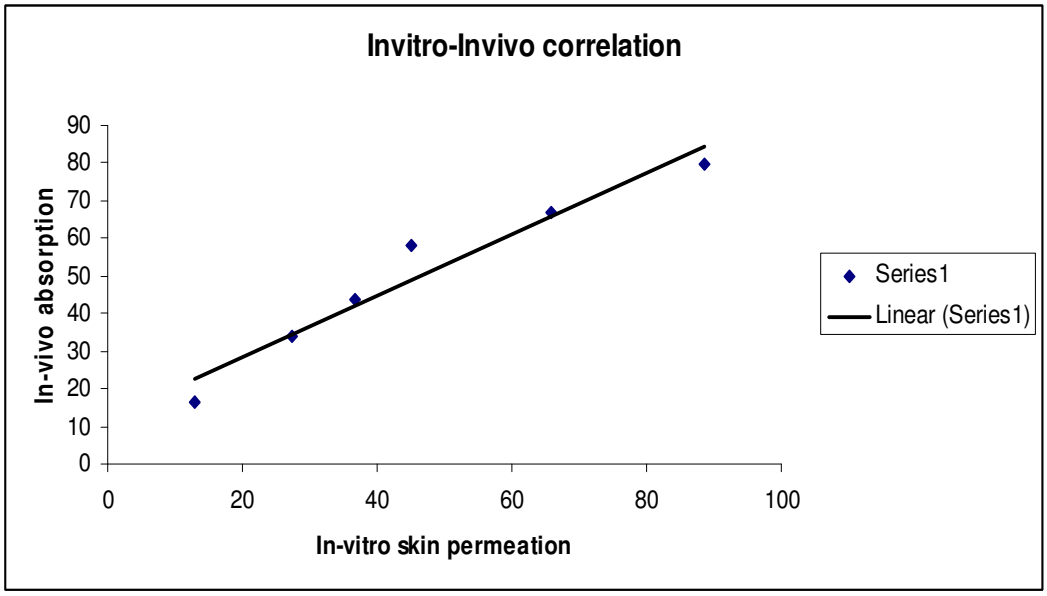


Fig: 73 Linear **curve for *In vitro/In vivo correlation.***

By establishing a relationship, between the *in vitro* permeation and the *in vivo* absorption parameters. The slope for these parameters is 0.814983. so, they are said to be near correlated, and the curves are not super imposable.

12. DISCUSSION

The topical and transdermal drug delivery system has gained wide range of applications in the field of drug delivery systems. The most common barrier for this type of system is to deliver the drug through skin via dermal and transdermal route.

The transdermal drug delivery system has gained popularity over the past few decades. Transdermal therapeutic system has generated an interest as this system provides the considerable advantage of a non-invasive parenteral route for drug therapy, avoidance of first-pass, gut and hepatic metabolisms, decreased side effects and relative ease of drug input termination in problematic cases. The major penetration pathway of drug molecules through the stratum corneum of intact human skin is by diffusing through the lipid envelopes of the skin cells.

The Nucleoside analog reverse transcriptase inhibitors have become the first-line therapy in treating HIV patients. Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. It improves the seroconversion of e-antigen positive hepatitis B and also improves histology staging of the liver. Long term use of lamivudine unfortunately leads to emergence of a resistant hepatitis B virus (YMDD) mutant. Despite this, lamivudine is still used widely as it is well tolerated.. Most of the Nucleoside analog reverse transcriptase inhibitors inhibitors are bi-peptides that are too hydrophilic to dissolve and penetrate through the lipid layers. Lamivudine was selected among the Nucleoside analog reverse transcriptase inhibitors due to the molecular size, therapeutic dose, half-life and to have a chronic treatment for the disease.

Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. It has been widely used for the treatment of HIV and Hepatitis B. The drug is considered a drug of choice in anti-retroviral therapy due to its effectiveness and low toxicity. Lamivudine shows 80% bio-availability, but presence of food reduces the Cmax and tmax, there is no change in its bioavailability. The sustained release of drug cannot be obtained with the oral administration and a chronic treatment is necessary for the HIV disease. Therefore,

the use of transdermal drug delivery system can reduce the side effects associated with conventional dosage forms and the frequent of dose administration can be reduced.

The preliminary study conducted on compatibility between Lamivudine, HPMC and EC revealed that there is no interaction between the drug and polymer.

Dose designing

Drug input rate across the skin was calculated to be 0.2067 mg/hr (Table-9). Therefore, the total drug required for 24 hr drug delivery is 5.0 mg.

THE TRANSDERMAL PATCHES

The polymers are the backbone for transdermal delivery. Some of the widely used polymers for the fabrication of transdermal patches are Cross-linked poly ethylene glycol (PEG) networks, Acrylic-acid matrices, Ethyl cellulose (EC), polyvinylpyrrolidone (PVP), Hydroxy propyl methyl cellulose (HPMC), Organogels, Ethyl vinyl acetate (EVA) co-polymers, and Chitosan etc. Among these polymers, HPMC and EC was selected for preparation of patches. Since, HPMC and EC are used as rate controlling polymers for sustained release and also they acts as stabilizing agent and having the property to inhibit oxidation. Hence, they are commonly employed in formulation of patches.

The Physico-chemical characteristics such as thickness of the patch, folding endurance, percentage of moisture absorbed, percentage of moisture lost, and water vapor transmission rate were found to be within the acceptable limits. The Patches were found to stable to withstand the stress.

Stability studies: All the patches were found to be most stable as per ICH guidelines.

***In vitro* skin permeation studies of Transdermal Patches:**

The results of the *in vitro* skin permeation profile demonstrated that duration of sustained release of the patches appears to depend on characteristics and concentration of HPMC and EC. As the concentration of HPMC and EC increased, the cumulative

percentage sustained releases were FPE_1 (80.840±0.894), FPE_2 (88.706±0.894), FPE_3 (57.265±0.894), FPE_4 (65.786±0.892) for 24hrs respectively with permeation enhancer patches (Fig. 63), the cumulative % sustained releases were F_1W (51.894±0.858), F2W (56.753±0.542), F3W (39.317±0.043), F_4 W (46.358±0.529) for 24hrs respectively without permeation enhancer patches (Fig. 42). An ideal transdermal delivery system should show sustained release characteristics. Taken this into account, the formulation F_2 met the above objective and hence, it was selected for *in- vivo studies*.

The results indicated that span 80 greatly influenced the permeation of the drug through the skin. Thus, suggesting the use of permeation enhancer in transdermal drug delivery system. The permeation profiles of the patches (Table-26) showed the regression value greater for zero order release than the first order release and the diffusion release constant (n) value in korsmeyer peppa's plot was found to be in the range of **$0.5 < n < 1.0$** ,thus concludes the mechanism of drug release as zero order and non-Fickian release (Anomalous).

The *in vitro* skin permeation data analysis

The permeability parameters like steady-state flux (J), permeability coefficient (P), and enhancement ratio (E_r) were significantly increased in formulations containing permeation enhancers. The permeability parameters of different formulations are given in tables 27 & 28

Skin irritation test

Any topical application should not produce skin irritation. This study was carried out on rabbit skin; it is observed that the transdermal patches are free from skin irritation.

Statistical analysis by ANOVA

The statistical analysis of the cumulative percentage of drug permeation data showed that the formulations are having 'p' value < 0.0001. This result suggests that the prepared Transdermal patches are extremely significant for the approach.

The *in - vivo* drug absorption data analysis

The *in-vivo* studies are performed for the best formulation selected from the *in-vitro* skin permeation studies.i.e, (FPE_2) formulation was selected. The HPLC spectrophotometer is used for analysing the plasma samples. The *in-vivo* drug absorption studies (Fig. 64) reveals that there is a sustained release of drug into the rats systemic circulation of 79.5% of drug absorption was found after 24hrs.

In-vitro/In-vivo correlation

By establishing a relationship, between the *in vitro* skin permeation studies and the *in vivo* absorption studies (Fig. 73). If this relationship becomes linear with a slope of 1, then curves are super imposable, and there is a 1:1 relationship which is defined as point-to-point or level - A correlation.

In the present study the slope between the *in vitro* skin permeation studies and the *in vivo* absorption studies was 0.814983. So, they are said to be near correlated, and the curves are not super imposable and are not linear.

13. CONCLUSION

The prepared Transdermal patch showed good sustained release properties. The results of the present study demonstrated that Lamivudine and HPMC in the ratio 1:5 (FPE_2) showed a good sustained release characteristic with the 3% Span 80 as a permeation enhancer, thus providing a better way of preventing first pass metabolism, frequent administration of drug and obtaining a sustained release effect for chronic treatment of HIV disease. The *in-vitro* skin permeation studies (FPE_2) showed a best sustained release effect over other formulations. The comparative statistical analysis done by ANOVA method proved that all the formulations are extremely significant for the Transdermal route. The *in-vivo* studies (FPE_2) also showed a sustained release effect of the drug into the systemic circulation. The *in vitro /in vivo* correlations are found to be not super imposable and said to be near correlated. Further *in-vivo* studies in detail and extrapolation of the data will help in development of a transdermal patch for clinical use.

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